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THE NATURE OF RESISTANCE OF FLAX TO MELAMPSORA LINI [1]

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INTRODUCTION

It has been recognized for some time that the culture of flax, *Linum usitatissimum* L., may in certain years be seriously impaired by flax rust (*Melampsora lini* (Pers.) *Lév.*). *Melampsora lini* has been reported under one name or another from North America, South America (Argentina), Europe (Great Britain, France, Germany, Russia, Italy, Bulgaria, Austria, Switzerland, and Spain), Asia (India, Asia Minor, and Siberia), Australia, China (Manchuria and Mongolia), and Japan (Sakhalin, Hokkaido, and Northern Honshu) (*20*).[3] Flax rust may therefore be considered to occur wherever flax is grown.

In the United States flax rust has been reported to be epidemic in the seed-flax regions of Minnesota, North Dakota, South Dakota, Iowa, Montana, and Wisconsin,[4] while it has been reported as endemic in the fiber-flax regions of eastern Michigan and the Willamette Valley of Oregon (*35*). In Canada the disease has been reported from the seed-flax centers of Alberta, Manitoba, Saskatchewan, Ontario, and Quebec.

Flax rust generally causes a partial or complete defoliation of seed-flax stands and in most cases ultimately causes a considerable reduction in yield. Most of the North American varieties of seed flax are not, as a rule, killed by rust under field conditions, although a few of the imported selections may be completely destroyed. In general, the average annual loss of seed flax from flax rust in North America varies from 1 to 10 percent of the total seed crop, although in 1928 losses as high as 35 percent were reported from several flax sections of North Dakota.

While various investigators have worked on the general aspects of flax rust, its epidemiology and control, little or no work appears to have been done on the nature of varietal resistance. The object

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[3] Reference is made by number (italics) to Literature Cited, p. 125.

[4] UNITED STATES DEPARTMENT OF AGRICULTURE, BUREAU OF PLANT INDUSTRY. FLAX. U. S. Dept. Agr. Bur. Plant Indus., Plant Disease Reptr. Sup. 35: 294–295, 1924; 40: 170–171, 1925; 48: 360–361, 1926; 53: 187, 1927; 62: 340, 1928; 71: 305, 1929. [Mimeographed.]

of this investigation was to determine, as far as possible, the exact nature of varietal resistance in cultivated flax to flax rust, with the aim of providing data which might be of assistance to plant breeders in the production of rust-resistant varieties.

During the past few years the nature of resistance of plants to certain fungus pathogens has been studied extensively, and three main theories of resistance have been developed: (1) The morphological theory, which affirms that certain morphological or anatomical features of the host plant either preclude the entrance of the pathogen into the plant or limit its development once it has gained entrance; (2) the functional theory, which suggests that stomatal movements of the host plant may play an important part in precluding or restricting the entrance of certain pathogens; and (3) the physiological theory, which postulates that certain inherent physiological properties of the host plant operate in determining the degree of resistance or susceptibility of the host to the fungus pathogen.

The present discussion approaches the problem from the angles suggested by the foregoing theories and is therefore divided into a morphological study, a stomatal study, and a physiological study.

REVIEW OF PREVIOUS INVESTIGATIONS

Arthur (*3, p. 201*), in 1907, demonstrated that *Melampsora lini* was euautoecious but did not find it specialized for particular species of *Linum*. Fromme (*14*), in 1912, described and figured the cytology of the causal organism, while Tobler (*42*), in 1920, studied the histological effects of the pathogen upon the host tissues. In Ireland the general aspects of flax rust have been studied by Pethybridge et al. (*26, 38, 39*). In 1926 Hart (*16*) studied the factors conditioning the development of flax rust. Henry (*19*), in 1928, studied the various phases of flax-rust control and demonstrated the possibility of control by the production of rust-resistant varieties through hybridization.

Bolley (*4, 5*), as early as 1903, noticed that several strains of cultivated flax reacted differently to flax rust, and obtained several rust-resistant strains by means of mass selection. Sydow and Butler (*41*) reported that European varieties of flax remained free from rust in India, while indigenous varieties were severely attacked. Eriksson (*12*), in 1912, noted that different varieties of flax had varying degrees of susceptibility. Butler (*6*) mentioned a variety of flax from Pusa, India, which had never been attacked successfully by flax rust. Westerdijk (*48*), in 1918, stated that flax rust was known in the Netherlands only on the white-blossom varieties.

Girola (*15*) pointed out that "Lino mal abrigo" (a group of Argentine varieties of flax having medium-sized seeds) was noted for its resistance to rust. Howard (*21, pp. 382–383*) reported that of the three classes of flax varieties, large-, medium-, and small-seeded, grown at Pusa, India, varieties of the last class did not suffer from rust. Dorst (*11*), in 1923, isolated rust-resistant strains of fiber flax in the Netherlands, and Dillman (*10*) has reported that certain varieties of Argentine flax are immune from rust when grown in the United States. Henry (*19*), working at the Minnesota station, conducted varietal tests for several years and noted distinct differences

in the resistance of different varieties. He isolated immune pure lines from commercial, large-seeded Argentine varieties, and also reported that the variety Ottawa 770B C. I. 355 was consistently immune in all of his tests. In addition, Henry found immune strains in the variety Williston Golden C. I. 25 and in several selections obtained from India.

COURSE OF DEVELOPMENT OF THE PATHOGEN IN SUSCEPTIBLE AND RESISTANT VARIETIES

DEVELOPMENT OF FLAX RUST WITHIN THE TISSUES OF A SUSCEPTIBLE HOST

While the sexual fusions and cytological phenomena of *Melampsora lini* have been investigated by Fromme (*14*), Moss (*36*, *37*), and Allen (*2*) a survey of the pertinent literature has failed to reveal a complete and detailed description of the histological development of the casual organism within the tissues of a susceptible host. Partly for this reason it was considered desirable to study histologically and depict diagrammatically the development of flax rust within the tissues of a susceptible host, namely, Winona flax. The development of the pycnial and aecial stages has been studied and illustrated in considerable detail by Fromme (*14*); hence, the writer studied the development of the pathogen from the time of penetration of the urediospore germ tube into the host until the formation of the morphologically mature telium.

Plants of Winona flax raised in pots in the greenhouse were inoculated by applying urediospores to the surfaces of the leaves and stems by means of a scalpel, and incubated for 48 hours. Leaf and stem material was removed from these inoculated plants at definite intervals and killed in medium chromo-acetic killing and fixing solution. The material was passed through the butyl alcohol series (*49*), imbedded, sectioned, and stained. The triple stain combination of safranine, gentian violet, and orange G employed by Wellensiek (*47*) was used.

Infection Phenomena

It has been shown by Hart (*16*) that the germ tubes of *Melampsora lini* grow toward the stomata of the host plant, form appressoria, enter through the stomatal pores, and subsequently develop substomatal vesicles which proceed to the formation of a mycelium. In this investigation attempts were made to obtain sections of stem material showing penetration of the urediospore germ tubes through stomata, but without success. However, definite cases of penetration into leaf material were readily obtained. By stripping off portions of the epidermis of inoculated leaves and staining with cottonblue stain it was possible to study the infection phenomena in surface view (fig. 1, *A*). The germ tube of the urediospore grows upon the surface of the leaf, in some cases branching rather frequently, but eventually some of the germ tubes grow over an open stoma. The tip of the germ tube, or the apical portion of a branch of the tube, in most cases swells to form a globoid, bladderlike structure, the "appressorium", situated immediately over the stoma. The function of the appressorium appears to be to provide, by virtue of its increased area of attachment, greater purchase for the germ tube while

the latter sends its infection hypha between the guard cells of a stoma. In cross section, the germ tube is observed to follow the contours of the leaf epidermis very closely (fig. 1, *B*). An infection peg is sent out from the appressorium and pushes its way between the guard cells of the stoma and swells to a small, globoid vesicle in the substomatal cavity, and then proceeds to the formation of an infection hypha.

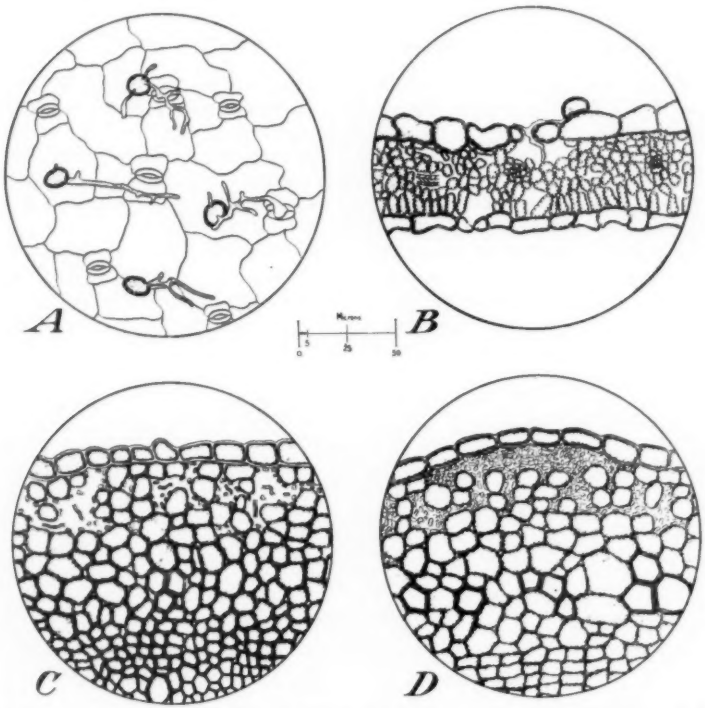

FIGURE 1.—Development of flax rust within the tissues of a susceptible host. (Camera lucida drawings): *A*, Surface view of leaf epidermis, showing germination of urediospores, appressorium formation, and penetration of germ tubes through stomata; *B*, transverse section of leaf, showing penetration of uredial germ tube through an open stoma and the formation of an infection hypha; *C*, transverse section of a stem, showing development of rust hyphae in cortical tissues of host; *D*, transverse section of stem 7 days after inoculation, showing formation of pustule stroma resulting in a bulging of the epidermis.

DEVELOPMENT OF THE UREDIUM

While definite cases of penetration were not obtained with stem material, very early stages of infection were observed. The first histological evidence obtained for infection in stem material was the occurrence of numerous isolated hyphae in the outer cortical tissues, appearing in transverse section as small spherical bodies (fig. 1, *C*). Approximately 7 days after inoculation a definite stroma is formed in the cortical tissues of the stem (fig. 1, *D*). The hyphae have greatly increased in number and have become aggregated

into a compact, stromalike body, which causes the epidermis to bulge noticeably. Approximately 10 days after inoculation a palisadelike layer of binucleate cells was observed immediately below the epidermis (fig. 2, *A*). The binucleate cells constitute the initial cells of the uredium and are supported by a stroma of small,

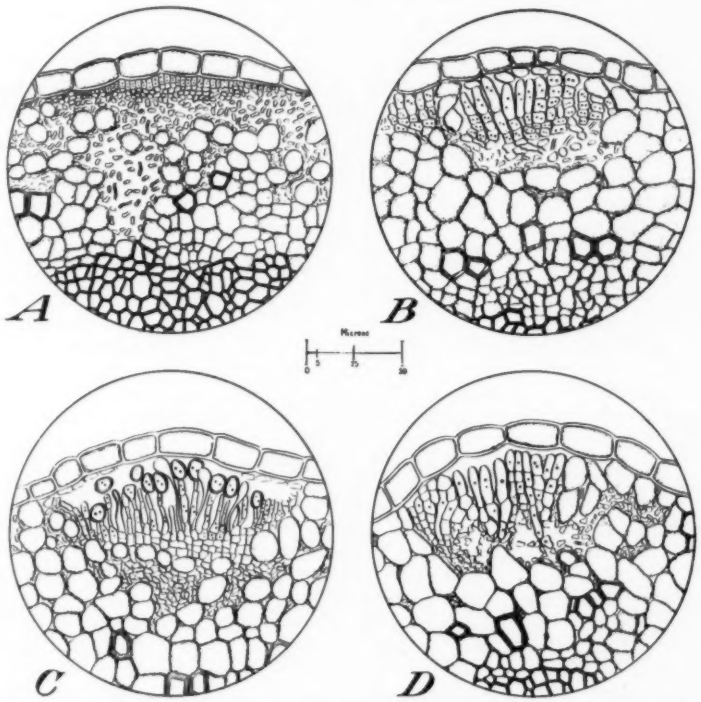

FIGURE 2.—Further development of flax rust within the tissues of a susceptible host. (Camera lucida drawings.) *A*, Transverse section of stem 10 days after inoculation, showing development of palisadelike layer of binucleate urediospore initial cells. *B*, transverse section of stem 12 days after inoculation, showing development of very young uredial sorus. The binucleate initial cells on the left side of the sorus have divided to give rise to the peridial, intercalary, and basal cells. On the extreme right of the sorus the basal cells have in turn divided to produce the apical spore initial cell, the still unelongated pedicel cell, and the basal mother cell. The evanescent peridial cells are also shown. *C*, transverse section of mature uredial sorus, showing fully formed urediospores with echinulate walls attached to the elongated pedicel cells. Intermingled with the pediceled urediospores the capitate paraphyses may be observed. *D*, transverse section of stem 21 days after inoculation, showing initial of telial sorus in the form of a palisadelike layer of cells. In center of sorus the initial cells have divided to form the apical teliospores and the basal cells.

rounded, fairly closely aggregated cells. In this stage the rust mycelium has become quite extensive and occurs throughout the cortical region of the stem, in some cases penetrating as far as the vascular ring, but in no case was it observed to affect or penetrate the vascular system. Approximately 12 days after inoculation the binucleate cells had developed into mature urediospores. Each cell of the palisade layer divided into three cells, the peridial, intercalary, and basal cell. The basal cell divides and thus gives rise

to spore initials, which, by their continued growth, result in the destruction of the intercalary cells. The peridial cells are usually evanescent but may persist under the still unruptured epidermis as scattered cells. The basal cell now divides twice, giving rise to the spore initial, the spore pedicel cell, and the mother basal cell. A uredium in this stage of development is illustrated in figure 2, *B*. Immediately beneath the epidermis five peridial cells are shown, the intercalary cells having disappeared. On the right of the uredium the three basal cells illustrated have completed their divisions, producing the apical spore initial, the still unelongated spore pedicel cell, and the mother basal cell. The six initials on the extreme left of the uredium are slightly less mature and the spore initials have not as yet commenced to round up. All the cells at this stage of development are binucleate. In the mature uredium the urediospores have developed their echinulate outer walls and are attached to the greatly elongated pedicels (fig. 2, *C*). Intermingled with the urediospores are the long, slender, capitate paraphyses, which tend to arch over the spores. In this stage the stroma is well developed and the epidermis greatly bulged. The spore pedicels continue to elongate and the spores to increase in size until sufficient pressure is exerted to rupture the epidermis and allow the spores to escape. In certain preparations it was noticed that many of the spores were flattened in contact with the epidermis, indicating that considerable pressure must have been applied before the epidermis was ruptured and the urediospores liberated.

Development of the Telium

Approximately 3 weeks after inoculation the initial of the telium makes its appearance. The telium may be initiated in an old uredium or it may arise independently in the cortical (or mesophyll) region of the host stem (or leaf). The telium arises in a manner similar to the uredium, small primordia developing below the epidermis and dividing to form a palisade of vertically arranged hyphal cells (fig. 2, *D*, extreme right). The initial cells are binucleate and become divided by a transverse septum into the lower basal cell and the upper teliospore (fig. 2, *D*, center of sorus). The teliospores become laterally compacted into a waxy sorus, which may become very extensive, a single telial layer in some cases extending half-way round the stem (fig. 3). The individual teliospores are embedded at their tips in a deep-staining waxy material, and quite commonly a second layer of teliospores is initiated outside the original layer (fig. 3). The apical teliospore elongates greatly and increases in size, while the basal cells apparently grow little or not at all. The teliospore apparently retains its binucleate condition until it is almost mature. Very clear cases of nuclear fusion in the teliospores were observed, and one such case is illustrated in figure 3. The teliospores to the left of this sorus are apparently still immature and consequently still binucleate. In these immature spores the nuclei are relatively small, in certain spores being rather widely separated, while in others they lie side by side. In several of the spores illustrated the two nuclei appear to have just commenced to fuse, forming a dumbbell-shaped structure, although the presence or absence of a separating membrane could not be determined. In the center of the sorus the spores possess a single large nucleus, which apparently has resulted from the fusion

of the two nuclei in the immature teliospore. These repeated observations would indicate that the spores in the center of the sorus attain maturity first and that maturity progresses centrifugally in the

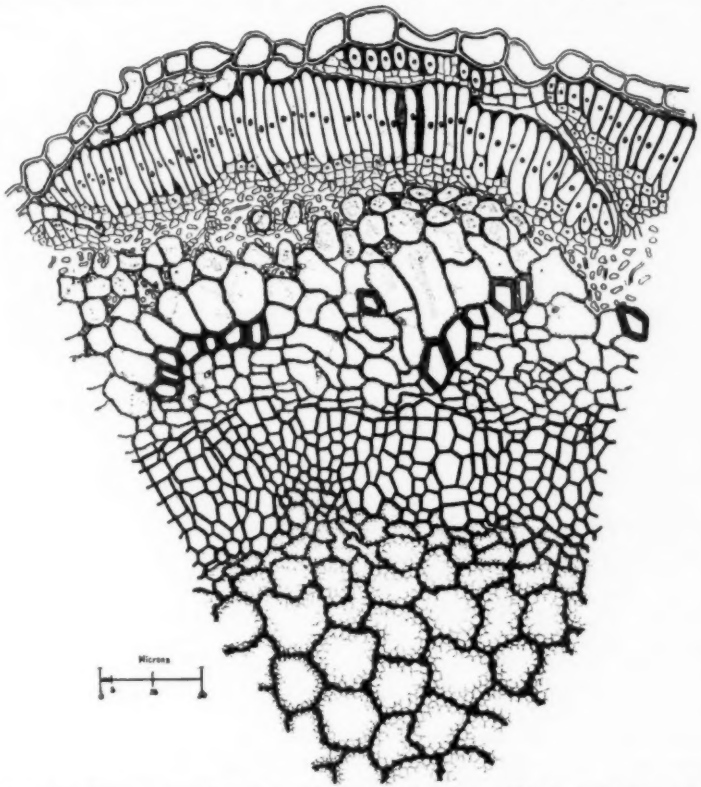

FIGURE 3.—Transverse section of a mature telium in a susceptible host, showing a band of teliospores embedded in a waxy sorus. On the left of the sorus the teliospores are still immature and binucleate. The teliospores in the center are mature and possess a single large fusion nucleus. Note development of secondary layer of teliospores immediately beneath the still unruptured epidermis. (Camera lucida drawing.)

telium. In this stage of development approximately half of the cortical region of the stem is occupied by the telium and its accompanying hyphae, and eventually the entire stem may become encircled by a layer of laterally compressed teliospores. The epidermis covering the telium apparently is not ruptured until the following spring when the teliospores commence to germinate.

HISTOLOGICAL STUDY OF UREDIAL TYPES

During the summer of 1932 the field reaction to rust of over 100 selections of flax [5] was determined in the flax-rust nursery on peat soil at Coon Creek, Anoka County, Minn. These observations were

[5] Material obtained from A. C. Dillman, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

made as part of another project[6]; hence, the detailed reactions of all of the selections are not given in this paper, although the reactions of the varieties recorded are based on these observations. It is possible that physiologic specialization exists in flax rust and that in other parts of the country the field reactions are not the same as at Coon Creek.

Under field conditions flax may react in five different ways to the flax-rust organism, depending upon the variety concerned. A variety may be immune, highly resistant, resistant, incompletely susceptible, or completely susceptible. The reaction types, erected on the basis of the uredium, have been designated respectively, 0, 1, 2, 3, 4. It was thought that a histological study of the different types of uredia might cast some light upon the problem of varietal resistance to flax rust.

Accordingly, rusted flax material was collected from the field plots, and uredia typical for the above-mentioned reactions were studied histologically. The material was killed in medium chromo-acetic killing solution, dehydrated in the butyl alcohol series, embedded in paraffin, stained with the triple combination of safranine, gentian violet and orange G, and sectioned longitudinally.

Type 0; immune.—Flax varieties immune from rust, such as Rio C. I. 280, Walsh C. I. 645, and Minnesota Selection C. I. 661, in general, show no symptoms whatever of the presence of the flax-rust organism within their tissues.

Type 1; highly resistant.—Flax varieties highly resistant to rust, such as Lino Grande C. I. 381, Bolley Golden C. I. 644, and Indian Type 55, are characterized in their field reaction to rust by the occurrence of necrotic flecks on the surface of infected leaves and by the absence of urediospore formation. When such areas are examined histologically it is found that the host cells in this region are completely broken down and replaced by dark-staining masses of necrotic tissues (fig. 4, *A*). It is rather remarkable that fungus hyphae were not observed among the remnants of the host cells, except at the extreme margins of the necrotic area. There is no indication whatever of the formation of a typical uredium, and all of the host tissue, including the epidermal cells in the vicinity of the infection zone, have apparently been broken down and digested. In addition, the line of demarcation between healthy and necrotic tissue is extremely sharp, which forms a basis for interesting conjecture. In varieties that react to rust in this manner, it seems possible that the fungus establishes parasitic relations with the host cells and destroys the latter so rapidly that it ultimately defeats its own ends by starving, due to its own inability to develop sufficiently fast to keep ahead of the dissolution of the host cells. If such is the case, it is reasonable to suppose that the rust hyphae themselves would ultimately undergo digestion, which would account for their absence in the center of the necrotic areas. If the rust hyphae failed to develop fast enough to keep ahead of the breaking down of the host cells, and thus died from lack of nutriment, one would not expect to find such hyphae in the region bordering the necrotic zone, as actually are found.

A second hypothesis may, therefore, be advanced to explain the mechanics of this type of rust reaction. The rust fungus may suc-

[6] Conducted in cooperation with C. C. Allison at Coon Creek.

ceed in penetrating to the interior of the host and may successfully establish parasitic relations. For a brief period the parasite may derive sufficient nutriment from the association to allow for its limited development, but ultimately a toxin may be produced by the breaking down of the parasitized host cells. Such a toxin might well be injurious to the rust fungus and eventually result in the rust hyphae depending upon their own reserve food material for existence after the supply of available food material from the host had been exhausted. Such a condition would eventually result in the death of the pathogen, and the net result would be the occurrence of an area in the leaf composed of broken-down host tissue with a few isolated rust hyphae at its extremity that had survived

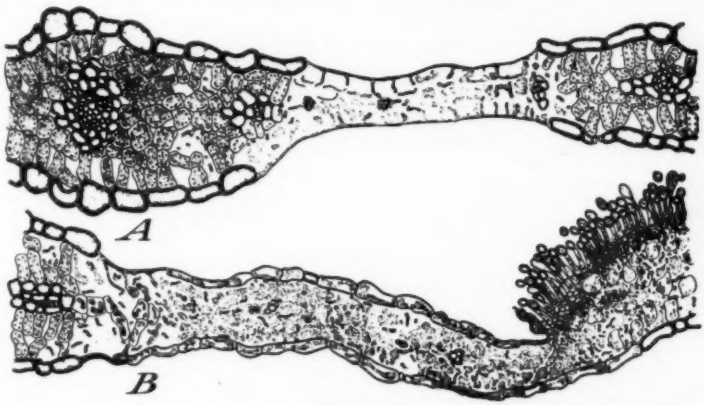

FIGURE 4.—Uredial pustule types. (Camera lucida drawings.) *A*, Highly resistant, type 1, pustule on Lino Grande flax. Note complete necrosis of host tissue in the infection zone and the absence of urediospore production. *B*, Resistant, type 2, pustule on the flax variety North Dakota Resistant 112; uredial pustule surrounded by a zone of completely broken-down host tissue.

but were incapable of causing further injury to the host. Such a phenomenon would result in the production of a rust reaction similar to that characteristic of type 1.

Type 2; resistant.—Certain varieties of flax, as, for example, Pale Pink (Brine) C. I. 649, Tammes Pale Blue C. I. 333, North Dakota Resistant C. I. 112, and Minnesota Selection C. I. 719 produce type 2 uredia. Externally the type 2 uredium closely resembles the type 1, consisting mainly of a necrotic zone, which, however, upon closer examination is found to possess a small group of urediospores in the center of the necrotic region. When viewed in longitudinal section (fig. 4, *B*) the small uredium is found to be encircled by a fairly wide zone of broken-down host tissue. In this region the mesophyll cells of the host have become transformed into irregular, dark-staining masses, scattered throughout which may be observed hyphal segments of the rust fungus. Contrary to the situation in the highly resistant type 1, the epidermal cells of the host in the type 2 uredium are not destroyed, although they are heavily parasitized and somewhat shrunken; they contain large haustoria of the rust fungus. In the region where the urediospores are produced the leaf is decidedly arched and greatly shrunken as compared with

healthy portions of the leaf. By far the greater part of this type of uredium is composed of dead masses of host cells, and the urediospores usually are quite limited in number and confined to a relatively small area in the center of the necrotic zone.

In this type of uredium a condition similar to that postulated for the type 1 may occur. The rust fungus apparently enters the host and parasitizes the mesophyll tissues of the leaf, upon which it lives and succeeds in developing a rather sparse hyphal system that extends only a relatively short distance through the host leaf system. Ultimately, either through lack of sufficient nutriment, or through the toxic effects of metabolic by-products arising from the breaking down of the host cells, the rust fungus dies, and its zone of activity is thus rather restricted and localized. However, in this type of reaction the rust fungus apparently succeeds in deriving sufficient food material from the association to enable it to produce relatively few urediospores before the delicate symbiotic balance between host and parasite is upset.

Type 3; incompletely susceptible.—A large number of flax varieties, e. g., Ottawa White-flowered C. I. 24, Indian Type 124, and Pale Blue C. I. 387, are characterized in their rust reactions by the development of uredia that are, however, surrounded by a well-defined chlorotic area. A longitudinal section of this type of uredium is illustrated in figure 5, *A*. It will be observed that there is no necrosis, and the fungus has succeeded in making considerable development and has culminated its activity by the production of numerous urediospores. However, in the immediate vicinity of the uredium, below the uredium, and within a narrow zone encircling it, the chloroplasts of the host cells have been destroyed, thus accounting for the chlorotic area surrounding the uredium, which is characteristic of this type of infection. The type 3 uredia are characteristically much smaller than the type 4, but they may break through both leaf surfaces, as illustrated in figure 5, *A*. Except for the smaller size and the presence of the chlorotic ring encircling them, they are in every way similar to the type 4 uredia. In this type of rust reaction, the symbiotic balance between host and parasite is not perfect, as is indicated by the destruction of the chloroplasts of the mesophyll cells. For this reason the reaction has been termed "incompletely susceptible."

Type 4, completely susceptible.—Varieties of flax completely susceptible to rust, such as the variety Winona C. I. 481, are distinguished by the production of large, extensive uredia that lack the chlorotic zone or necrotic tissue characteristic of varieties resistant to the rust. A longitudinal section of this type of uredium is illustrated in figure 5, *B*.

From the foregoing histological studies it is apparent that the relationship between flax rust and plants resistant to its attack is rather complex. It would appear from the indications obtained that the physiological make-up of the different varieties may influence strongly their reaction to rust, as is evidenced by the different types of uredia produced on different varieties. In addition, it is fully realized that it would be desirable to study histologically early stages of infection in varieties resistant to *Melampsora lini*, and studies of this nature have been undertaken.

UREDIOSPORE GERMINATION IN EXTRACTS FROM FLAX VARIETIES DIFFERING IN REACTION TO RUST

In the histological study of the different types of uredia produced on varieties differing in their reaction to rust, indications were obtained pointing to the fact that differences in the physiological properties of the different varieties might be responsible in part for the different rust reactions as expressed by the type of uredium developed. It was thought logical at this point to approach the problem from the physiological aspect of plant-disease resistance.

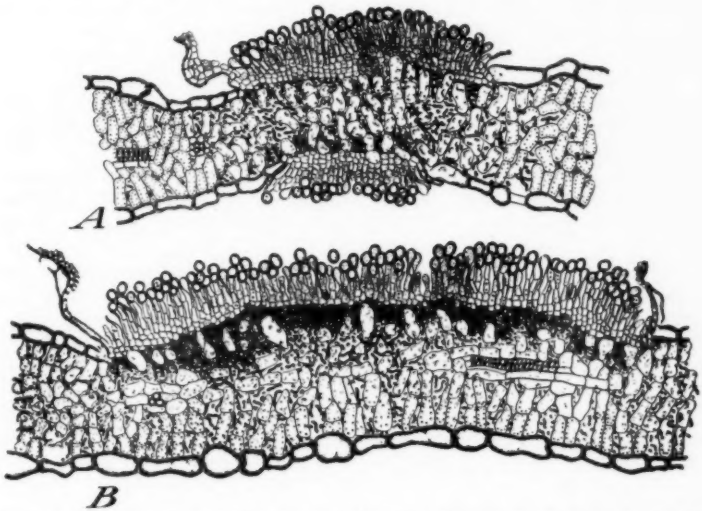

FIGURE 5.—Other uredial pustule types. (Camera lucida drawings.) *A*, Incompletely susceptible, type 3, pustule on Buda flax. Note the area immediately surrounding the pustule in which the host cells are devoid of chloroplasts. *B*, Completely susceptible, type 4, pustule on Winona flax. Note the extensive uredial pustule produced without necrosis or chlorosis of the host cells. The host cells immediately below the pustule still retain their chloroplasts and the rust hyphae ramify extensively throughout the host tissues.

The concept of the physiological nature of disease resistance is founded on the hypothesis that resistance is partly due to differences in the value of different hosts as substrates for the vegetative growth of the pathogen concerned. Ward (*44*, *45*, *46*), on the basis of investigations concerning the parasitic relationships between the bromegrasses and their rust (*Puccinia dispersa* Eriks. and Henn.), advanced the theory that differences in rust reaction might be due to materials present or produced in resistant plants, but absent in others, that inhibited the growth of the parasite. Leach (*28*) has advanced the theory that materials furnishing nutrients necessary to the development of the stem rust fungus may be present only in susceptible plants and absent in plants resistant to or immune from this fungus. Ezekiel (*13*), from the results of extensive investigations on the nature of physiologic resistance to *Puccinia graminis tritici*, concluded that extracts obtained from different wheat varieties differed in their ability to support the growth of

physiologic forms of stem rust in precise agreement with the respective resistance or susceptibility of the variety to the physiologic forms concerned. Compatibility of physiologic forms with the host varieties was indicated almost universally by greater lengths of urediospore germ tubes in extracts from susceptible varieties, less branching and more infrequent production of apical swellings than in extracts obtained from resistant varieties. Increase in length of the germ tubes was obtained in extracts derived from rusted plants as compared with the germination in extracts obtained from healthy wheat plants. Extracts stored in the refrigerator for 8 months continued to differentiate between forms to which the variety concerned was susceptible or resistant. Extracts prepared by methods involving the application of heat apparently did not contain the material or materials affecting germ-tube elongation.

During the summer of 1933 flax material was collected from healthy flax plants in early stages of maturity growing in the flax-rust nursery at Coon Creek and at University Farm, St. Paul, Minn. A definite amount of each variety was weighed out, and then all parts of the plant—stem, leaves, sepals, and blossoms—were ground up in a meat grinder. The ground-up material was steeped in two and one-half times its own weight of sterile distilled water and placed in the refrigerator for 2 hours. The plant juice was then extracted by squeezing the material through several thicknesses of cheesecloth and then by means of pressure in a hydraulic press equipped with press cups of noncorrosive monel metal. The expressed juice was then filtered twice through filter paper in a Büchner funnel and stored in sterile flasks in the refrigerator at 10° C. The concentration of an extract prepared by this procedure is equivalent to a concentration of 0.4, or a 40-percent solution. In all cases urediospore germination studies were made within 24 hours after the extract was prepared. The 40-percent stock solution was diluted with sterile distilled water to concentrations of 10, 1.0, 0.5, and 0.1 percent. Hanging drops of each extract were made up on sterile cover glasses supported on glass rings contained in a Petri dish lined with moistened filter paper. All glassware used in these experiments was thoroughly cleansed in potassium dichromate-sulphuric acid cleaning solution, washed in alcohol, rinsed in distilled water, and finally sterilized in the hot-air oven at a temperature of 150°. Hanging drops were made up in quintuplicate for each dilution of each extract, and these were inoculated by brushing freshly gathered urediospores on the surface of the drop by means of a stiff camel's-hair brush. Germination counts were made at 12-, 24-, and 36-hour intervals, the individual results given in the following tables being the averages obtained from the five hanging-drop cultures of each extract, that is, of approximately 500 spores. The length of the germ tubes was determined by means of an ocular screw micrometer, the results in tables 1 and 2 being the average of at least 10 determinations for each extract. The length of the longest branch was used as a criterion of length, and the results given were carefully checked by visual observation of the five hanging drops. Check cultures were made up in sterile distilled water. The Petri dishes containing the hanging-drop cultures were incubated in controlled soil-temperature tanks adjusted to run at a temperature of 19°.

TABLE 1.—*Germination of urediospores after 12 and 18 hours in 10-percent extracts from varieties of flax differing in their reaction to rust*

Source of extract [1]	Rust reaction [2]		Germination and length of germ tubes			
			12 hours		18 hours	
	Prevalence	Type no.	Germination	Length	Germination	Length
	Percent		*Percent*	*Microns*	*Percent*	*Microns*
Distilled water			55	345	55	380
Winona C. I. 481	90	4	42	257	63	328
Bison C. I. 389	1–15	3–4	43	289	50	318
Redwing C. I. 320	15	1	36	291	37	292
Pale Blue C. I. 176	15	1	16	301		
Walsh C. I. 645	0	0	7	173	13	255

[1] Extracts from healthy plants.
[2] Based on Coon Creek observations, 1932.

At the outset a preliminary experiment was made to determine whether or not extracts from different flax varieties varied in their ability to support the vegetative growth of urediospores of *Melampsora lini*. Samples of flax material were collected from healthy plants of Winona, Bison, Redwing, Pale Blue, and Walsh flax growing in the rust nursery at University Farm and were extracted by the methods previously outlined. The stock solutions were diluted with sterile distilled water to a concentration of 10 percent of the natural strength of the plant juice. Hanging drops made up in quintuplicate were inoculated with a bulk sample of urediospores freshly gathered from the field plots and were incubated at 19° C. The experiment was repeated several times to ensure uniformity of technic, observations being made in each case. The experiment was finally set up, freshly prepared extracts from the different varieties being used. The results obtained are given in table 1. These results indicate clearly that the ability of the extracts to support the growth of flax rust urediospores varies in accordance with the resistance or susceptibility of the varieties from which they were obtained. The reduction in percentage germination below the check culture (distilled water) was particularly striking in extracts obtained from the highly resistant variety Pale Blue and the immune variety Walsh. The germ-tube length in extracts obtained from the variety Walsh was considerably reduced when compared with the check culture in sterile distilled water, but the most striking difference was in the nature of the germ tubes in the different extracts.

In sterile distilled water the germ tubes were very irregular and greatly branched (fig. 6, *A*), similar to urediospore germ tubes of *Puccinia graminis tritici* described by Ezekiel (*13*). In extracts obtained from the susceptible variety Winona the germ tubes were strong and regular, and the branching was confined to the end of the germ tube (fig. 6, *B*). In extracts obtained from highly resistant varieties, such as Redwing and Pale Blue, the germ tubes were inclined to be irregular and coiled (fig. 6, *D*). In extracts from the immune variety Walsh the germ tubes were extremely irregular and branched and were inclined to coil rather tightly (fig. 6, *E*).

From the results obtained in this preliminary experiment, it was concluded that there are differences in the ability of plant extracts from flax varieties differing in their field reaction to rust to support the vegetative growth of urediospores of *Melampsora lini*.

A second experiment was conducted to determine the effect of dilution of extracts from different flax varieties on their ability to support the growth of urediospore germ tubes. Extracts were obtained from several flax varieties as in the previous experiment, except that rusted as well as healthy Winona plants were used for extraction. The extracts were handled in exactly the same way as in the previous experiment, the stock solutions being diluted

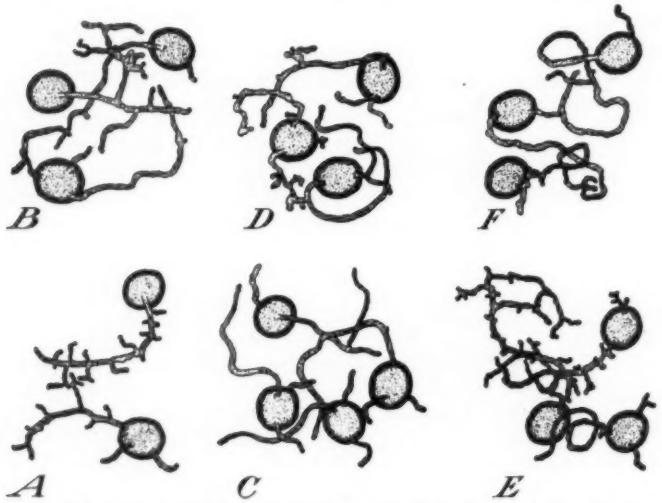

FIGURE 6.—Effect on the germination of urediospores of plant extracts from flax varieties differing in their reaction to rust: *A*, Germination in sterile distilled water; *B*, germination in extracts obtained from healthy plants of the completely susceptible variety Winona; *C*, germination in extracts obtained from rusted plants of the completely susceptible variety Winona; *D*, germination in extracts obtained from the resistant variety Redwing; *E*, germination in extracts obtained from the immune variety Walsh; *F*, germination in extracts obtained from the immune variety Rio.

to concentrations of 5 and 1 percent. The results of this experiment are given in table 2. The percentage germination in the different extracts agrees fairly closely with the results obtained in the previous experiment, the different extracts supporting the growth of urediospore germ tubes in accordance with the resistance or susceptibility to rust of the varieties from which they were obtained. No apparent differences appeared in this experiment between the percentage germination of urediospores in extracts obtained from healthy and from rusted Winona plants. However, the rusted plants from which the latter extract was obtained were only in the initial stages of uredium formation and were not heavily infected. Differentiation in germ-tube length was much sharper in this experiment than in the previous one. The length of the germ tubes was exceptionally low, for some reason, in sterile distilled water, but they were very irregular, as previously described. Germ-

tube length was again considerably reduced in extracts from the immune variety Walsh, the nature of the germ tubes being the same as in the previous experiment (fig. 6, *E*). The nature of germ-tube growth in extracts from rusted Winona plants is illustrated in figure 6, *C*. The concentration of the plant extract appeared to affect germination but little.

TABLE 2.—*Effect on urediospore germination of extracts of different percentage concentrations from varieties of flax differing in their reaction to rust*

1- AND 5-PERCENT CONCENTRATIONS

Source of extract [1]	Rust reaction		Concentration of extract	Germination and length of germ tubes			
				12 hours		36 hours	
	Prevalence	Type no.		Germination	Length	Germination	Length
	Percent		*Percent*	*Percent*	*Microns*	*Percent*	*Microns*
Distilled water				46	153	54	
Winona C. I. 481 (rusted)	90	4	5	42	253		
			1	41	282	46	
Winona C. I. 481	90	4	5	35	264		
			1	40	286	48	
Bison C. I. 389	1–15	3–4	5	23	276		
			1	28	325	31	
Redwing C. I. 320	15	1	5	37	200		
			1	32	207	37	
Walsh C. I. 645	0	0	5	14	195		
			1	14	173	17	

1- TO 0.1-PERCENT CONCENTRATIONS

Distilled water				39	213		234
Winona C. I. 481 (rusted)	90	4	1.0	16	212	16	
			.5	38	357	34	381
			.1	34	282	28	278
Winona C. I. 481	90	4	1.0	14	161		
			.5	19	295	26	300
			.1				
Rio C. I. 280	0	0	1.0	13	191	14	
			.5	8			
			.1	27	338	20	332

[1] Extracts from healthy plants except where indicated.

In the previous experiment the results indicated that no differences existed in urediospore germination in 5- and in 1-percent extracts. Another experiment was therefore set up to determine whether extreme dilution of the extracts would affect urediospore germ-tube growth. Extracts were made from the immune variety Rio and from healthy and heavily rusted Winona plants, the procedure of extraction being exactly the same as in previous experiments, the stock solutions being diluted with sterile distilled water to cencentrations of 1.0, 0.5, and 0.1 percent of the initial concentration of the plant juice. From the results in table 2 it is apparent that decided differences existed in the germination in different extracts. In extracts obtained from the immune variety Rio, the percentage germination after 12 hours' incubation was considerably reduced as compared with the check series in sterile distilled water. Furthermore, extreme dilution of this extract (0.1 percent) appeared to lessen its inhibitory effect, the germination of 13 percent in the 1-percent

extract as compared with 27 percent in the 0.1-percent extract appearing to be significant. The germination of 8 percent in the 0.5-percent extract is difficult to explain, but was probably due to experimental error. The extract from rusted Winona plants stimulated the percentage germination to a certain degree; the high concentration had an inhibitory effect, the optimum concentration being 0.5 percent. The results of measuring the length of the germ tubes substantiated the percentage germination counts, stronger extracts from the immune variety Rio reducing the germ-tube length, and stimulating it in extracts of 0.1-percent concentration as compared with the check cultures in sterile distilled water. In 0.5 percent extracts obtained from Winona flax plants the germ tubes were significantly longer than in distilled water, germ-tube length being greater in extracts from rusted plants than in extracts from healthy Winona plants. Percentage germination counts and germ-tube lengths were also determined at the end of 24 hours' incubation, and in general the results check reasonably well with the determinations made at the end of 12 hours' incubation. The gaps in the table are due to contamination of the cultures and also to the fact that excessive coiling of the germ tubes in many cases made it impossible to estimate accurately germ-tube length.

The differences in germination in extracts from healthy and rusted Winona plants are inconclusive in view of the negative results obtained in the initial experiment, and further investigations on this point will be necessary.

From the foregoing experiments it was concluded that extracts obtained from flax varieties differing in reaction to rust differ in their ability to support the growth of urediospore germ tubes in accordance with the resistance or susceptibility of the varieties from which they were obtained. Such differences are expressed in the percentage germination, in the length of the germ tubes, and in the nature of the germ tubes in the different extracts.

RELATION OF MORPHOLOGY OF THE HOST TO RESISTANCE

In recent years the nature of morphological resistance to certain diseases has been studied extensively, and the accumulated literature is voluminous. The possible exclusion of parasites by means of unusual thicknesses of cuticle, by the development of layers of corky cells, and by the presence of unusually tough membranes has been suggested independently by several workers as possible factors in determining resistance. Extensive investigations have been made of morphological resistance in cereal rusts, and it has been shown by Hart (*17*) and others that the mycelium of *Puccinia graminis tritici* is limited within the host plant entirely to the chlorenchymatous tissue, the sclerenchymatous tissue remaining impervious to attack. Hart concluded from her studies of the morphology of the peduncle of numerous wheat varieties that wheats generally resistant to stem rust in the field possessed more sclerenchyma and less parenchyma than the highly susceptible varieties. The size, shape, and arrangement of the parenchyma strands in the peduncle proved to be important in determining the spread of the rust mycelium and also in limiting the size of the uredia. The same conclusions were arrived at by Hursh (*22, p. 407*), who states:

In those varieties, therefore, in which there is a large amount of collenchyma, large uredinia are likely to be produced, while in those varieties in which the collenchyma bundles are small, the uredinia are likely to be narrowly linear. Varieties in which there is a great deal of sclerenchyma are likely to be injured less by the rust, as there is a mechanical limitation to the spread of the mycelium.

Thus, in the light of our present knowledge, morphological resistance in wheat to stem rust appears to be due to the large amount of sclerenchyma in proportion to the amount of parenchyma present in the leaf, stem, or peduncle of the variety concerned.

MORPHOLOGY OF THE FLAX LEAF

The morphology of the flax leaf was studied by sectioning and staining leaves of several varieties of flax. The epidermis of the flax leaf is composed of irregularly rectangular cells, the cells of the lower epidermis being in most cases considerably larger than those of the upper epidermis (fig. 1, *B*). There is a rather indefinite palisade layer of loosely arranged, rectangular cells situated immediately beneath the epidermis. The mesophyll consists of loosely arranged, approximately spherical cells containing numerous chloroplasts around their periphery. The leaves of most flax varieties have a large vascular bundle associated with the somewhat prominent midrib, the other smaller vascular bundles lying parallel to the median one. Usually associated with the vascular bundles are a number of small sclerenchyma cells, but the formation of large sclerenchyma girders, as reported for wheat, was not observed in the leaves of any of the varieties of flax examined. The leaves of numerous flax varieties, differing widely in their reaction to rust in the field, were carefully examined histologically, but no striking major anatomical differences were observed, although minor variations, such as differences in the size and shape of the epidermal cells, were noted.

From these observations it was concluded that there are no major anatomical features of the flax leaf which could explain the various degrees of resistance and susceptibility of the different varieties to rust.

MORPHOLOGY OF THE FLAX STEM

For a study of the structure of the flax stem, several varieties of flax were grown in pots in the greenhouse and material was sectioned immediately prior to the flowering stage. For detailed morphological studies material was killed in Flemming's weaker solution (*8*), dehydrated in butyl alcohol (*49*), embedded, and stained with Flemming's triple stain (*48*). The butyl alcohol method of dehydrating was found to be superior to the ordinary alcohol-xylol series, causing less plasmolysis of the cells, producing less hardening of the stem tissues, and saving a great deal of time. Numerous staining combinations were used, but the triple combination of safranine, gentian violet, and orange G was most satisfactory. In addition, fresh material was sectioned freehand and stained with lactophenol cotton-blue stain (*30*). All measurements of epidermal cells were made from this material.

The flax stem is a hollow cylinder. It has an epidermis of varying thickness, composed of relatively thick-walled cells, which may

be rectangular or isodiametric depending upon the variety. Immediately beneath the epidermis is the cortical parenchyma, which varies in extent in different varieties and is composed of irregular cells arranged rather loosely. The outer cells of the cortex are usually chlorenchymatous and are limited internally by rather large, fairly regular, compactly arranged parenchyma cells which surround the cortical fibers. The vascular tissues in most of the varieties examined are arranged in a rather extensive continuous ring, but in some varieties they appear as isolated bundles. Internal to the vascular tissue is the inner parenchyma, comprising large regular cells surrounding the pith cavity.

From a study of the anatomy of the stems of various flax varieties certain generalized comparisons may be drawn between susceptible, resistant, and immune varieties. In the susceptible varieties examined, such as Crepitans and Winona, the epidermal cells in general are rectangular and fairly thin-walled, and there is no indication of extensive cuticular development (fig. 7, *A*). Many of the susceptible varieties possess a continuous ring of large, thick-walled cortical fibers, but the size and distribution of the cortical fibers do not appear to be consistently of this type in all susceptible varieties. In the variety Pinnacle, which is slightly less susceptible than Crepitans, the epidermal cells tend to approach the isodiametric condition, are slightly thicker-walled, and possess a visible but very thin cuticle (fig. 7, *B*). In such a variety there is also a tendency to develop a single layer of small, regular cells immediately below the epidermis. Observations indicate that this layer of cells comprises a collenchymatous hypodermis. In the variety Pinnacle this layer of cells is not continuous.

Resistant varieties, such as Bolley Golden C. I. 644 (fig. 7, *C*), Lino Grande C. I. 381, and Abyssinian C. I. 300, are characterized by large, almost isodiametric, thick-walled epidermal cells. The outer epidermal wall in most cases is much thicker than the inner cell wall, and in the resistant flax varieties examined it was covered with a fairly thick, well-defined cuticle. In these varieties also, immediately underlying the epidermis, a continuous layer of collenchymatous hypodermis one cell thick was present.

In immune varieties, such as Minnesota Selection C. I. 661 (fig. 7, *D*), Rio C. I. 280, Indian Type 46, and Walsh C. I. 645, the epidermal cells appear to be completely isodiametric, with a thick outer wall and a very thick cuticle, which in the variety Rio sometimes attains a thickness of 2μ to 4μ.

From the foregoing study of the morphology of the flax stem it is apparent that there are certain morphological differences between different varieties of flax, and it would seem likely that these differences may operate in determining resistance or susceptibility to flax rust, probably in conjunction with other factors.

THICKNESS OF THE EPIDERMIS IN RELATION TO THE FORMATION OF UREDIA

It is known from the investigations of Hart (*17*) that the size and shape of the epidermal cells, the thickness of the outer epidermal wall, and the presence of a cuticle play an important role in pustule formation in the cereal rusts. In varieties of flax susceptible to flax rust, such as Winona, the fungus apparently encounters little difficulty

in rupturing the epidermis, as numerous large uredia develop. In resistant and partially resistant varieties, such as Buda, Lino Grande, and Bolley Golden, on the other hand, the uredia are generally smaller and fewer; and in some cases on the leaves the symptoms of

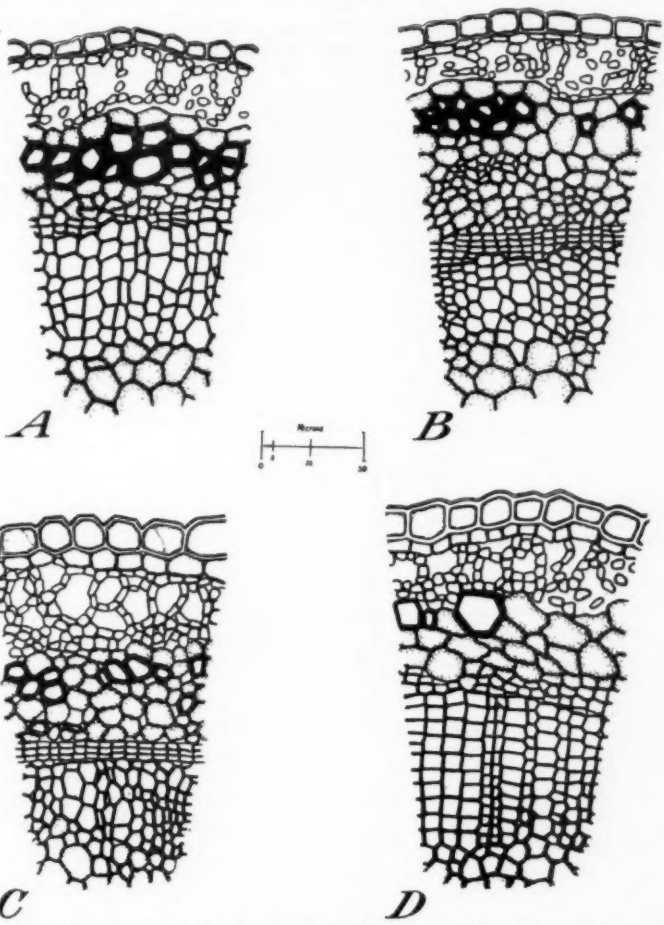

FIGURE 7.—Stem structure in flax varieties differing in their field reaction to rust. (Camera lucida drawings.) *A*, Variety Crepitans, completely susceptible; *B*, variety Pinnacle, partially resistant; *C*, variety Bolley Golden, resistant; *D*, variety Minnesota Selection C. I. 661, immune.

infection are confined to flecks on the upper leaf surface. In some cases, subepidermal pustules are developed on the stems of certain resistant varieties, apparently as a result of the rigidity of the epidermal membrane.

An analogous situation has been noted in wheat in connection with stem rust. Hart (*17*) observed that the epidermal membranes of

wheat varieties susceptible to stem rust (Little Club, Ruby, and Marquis) were comparatively thin and easily ruptured. The epidermis of some wheat varieties fairly resistant to stem rust (Khapli, Kota, and Webster), on the other hand, was found to be very thick as compared with that of the susceptible varieties, and in these varieties subepidermal pustules are commonly developed.

In this investigation, the thickness of the epidermal membrane of several flax varieties was determined by sectioning freehand fresh material, staining with lactophenol, cotton-blue stain, and then measuring with an ocular screw micrometer. The results, which are given in table 3, should be considered as relative and not in any way as absolute, for the thickness of the epidermal membrane undoubtedly varies considerably under different environmental conditions.

The immune varieties examined were found to have a very thick cuticle and a thick epidermal membrane. The individual cells of the epidermis were almost perfectly isodiametric, the ratio of length to width varying from 1.0:0.92 to 1.0:1.01. Resistant varieties had a slightly thinner epidermal membrane than the immune varieties examined, and they also had a thick cuticle. The epidermal cells were almost isodiametric, the ratio of length to width being about 1.0:0.8. Susceptible varieties possessed a relatively thin epidermis, a well-developed cuticle generally was absent, and the epidermal cells were elongate-rectangular, the ratio of length to width varying from 1.0:0.44 to 1.0:0.65. The results of these determinations would appear to indicate that the thickness of the epidermal membrane may play an important role in pustule formation.

TABLE 3.—*Dimensions of the epidermal cells of varieties of flax differing in their reaction to rust*

Reaction and variety [1]	Rust reaction		Length of epidermal cells	Width of epidermal cells	Ratio length to width	Cuticle
	Prevalence	Type no.				
Immune:	*Percent*		*Microns*	*Microns*		
Rio C. I. 280	0	0	22.9	23.2	1:1.01	Very thick.
Minnesota Selection C. I. 661	0	0	27.2	18.3	1:.67	Fairly thick.
Minnesota Selection C. I. 651	0	0	22.3	20.5	1:.92	Very thick.
Indian Type 46	0	0	20.8	19.2	1:.93	Thick.
Resistant:						
Lino Grande C. I. 381	Tr.	1	25.6	17.0	1:.66	Do.
M–25–221 C. I. 423	45	1	28.2	23.6	1:.83	Fairly thick.
Bolley Golden C. I. 644	50	1	22.5	17.7	1:.78	Do.
Indian Type 55	50	1	19.3	16.6	1:.86	Very thick.
Abyssinian C. I. 300	55	1	19.4	19.3	1:.99	Do.
Susceptible:						
Buda C. I. 326	85	3	24.4	15.9	1:.65	Thin.
Pinnacle C. I. 693	60	4	20.8	12.3	1:.59	Absent.
Crepitans C. I. 506	70	4	20.2	8.9	1:.44	Do.
Winona C. I. 481	90	4	27.0	15.2	1:.56	Do.
Deep Pink C. I. 648	90	4	22.8	15.6	1:.68	Very thin.
Indian Type 22	100	4	25.5	17.1	1:.67	Do.

[1] C. I. = Accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

RESISTANCE OF THE EPIDERMIS TO PUNCTURE

The ability of *Melampsora lini* to develop normal uredia varies greatly in the different varieties of flax. In completely susceptible varieties, such as Winona, characteristic type 4 uredia usually break through both leaf surfaces. In partially susceptible varieties, such as

Indian Type 12, Indian Type 55, and Lino Grande, a few well-developed uredia may occur on the lower leaf surface, but often there are none, and numerous hypersensitive flecks indicating resistance are present. It would thus seem reasonable to suggest that the tensile strength of the epidermal membrane may play some part in determining the ability of the fungus to form normal uredia on different varieties. Accordingly, the resistance of the epidermis of a number of varieties of flax to puncture was determined by means of a modified Joly balance.

The Joly balance was first adapted to the study of the resistance of various plant tissues to puncture by Hawkins and Harvey (*18*), the apparatus later being further modified by Melander and Craigie (*34*). As modified by the latter workers, the apparatus consisted of the ordinary Joly balance standard, but the pan of the balance was removed and replaced with a coupling designed to carry a carefully ground phonograph needle. The downward path of the needle was maintained in a vertical direction by means of a wire guide attached to the standard of the apparatus. In making a determination, the leaf material was placed on a paraffin block on the stage of the apparatus and weighted down by means of a lead weight provided with a slit to allow for the insertion of the needle. The needle was then brought to rest on the surface of the leaf material, and an indicator on the needle coupling was so adjusted as to correspond with a hair line etched on a small mirror placed immediately behind the needle coupling. The reading on the vernier scale was then recorded, and by decreasing the tension on the spring and closely observing the needle indicator through a magnifying glass, it was possible to observe the exact moment of puncture of the leaf epidermis, a perceptible drop in the needle being observed distinctly when this occurred. The reading on the vernier scale was again recorded, and the difference between the initial and final readings gave the distance it was necessary to lower the needle in order to effect penetration of the leaf surface. A fall of 1 cm on the vernier scale was found by experimentation to be equivalent to a pressure of 502 mg. The diameter of the needle used was approximately 25μ. Determinations were made for the upper and lower leaf surface—10 leaves being used for each variety—and 50 determinations were made for each leaf surface. To ensure uniform experimental conditions, one-half of the determinations on each variety were made in the morning and one-half in the afternoon, and approximately similar days were selected during the course of the work, which was done in the greenhouse. To ensure uniform leaf turgor, fresh leaves were removed from growing plants of the same age, and the determinations for each leaf surface were made immediately. As a rule, only five determinations were possible for each surface of a single leaf, as the leaf wilted soon after it was removed from the plant; the resistance of wilted leaves to puncture probably would be different from that of turgid leaves.

The results of the determinations are given in table 4. It is apparent that the epidermis of the susceptible varieties examined was far easier to puncture than that of resistant varieties. The leaf epidermis of the resistant varieties was in turn harder to puncture than that of immune varieties. In immune varieties, ap-

parently, the fungus fails to establish successful parasitic relations, whereas in resistant varieties it does appear to establish itself for a while within the tissues of the host, but the uredia subsequently developed are restricted in size and in some cases appear only on the lower leaf surface. The results presented in table 4 show a significant difference in the amount of pressure required to puncture the lower and upper leaf epidermis of resistant varieties, the upper epidermis being consistently more resistant to puncture, and this may account for the absence of uredia and the appearance of flecks on the upper leaf surface of the resistant varieties examined.

TABLE 4.—*Pressure required to puncture the outer epidermal wall of the upper and lower leaf surfaces of varieties of flax differing in their reaction to rust (as measured by the July balance)*

Reaction and variety	Leaf surface	Range	Mean	Standard deviation	Mean differences and probable errors of the differences between the two means
Immune:		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Minnesota Selection C. I. 664.	Upper	572.28–853.40	683.02±7.45	78.10±5.27	41.78±10.81
	Lower	512.04–903.60	641.24±7.83	82.13±5.54	
Minnesota Selection C. I. 661.	Upper	602.40–853.40	723.90±8.16	85.55±6.07	103.93±14.05
	Lower	351.40–903.60	619.97±11.44	120.00±8.10	
Indian Type 46	Upper	486.94–1,099.38	711.44±11.06	116.00±7.82	78.94±14.32
	Lower	476.90–903.60	632.50±9.10	95.60±6.50	
Resistant:					
Indian Type 12	Upper	476.90–1,014.04	708.22±11.68	132.90±8.96	76.79±15.57
	Lower	426.70–858.50	631.43±10.30	108.00±7.28	
Lino Grande C. I. 381	Upper	602.40–1,280.10	988.54±15.92	166.60±11.32	123.44±20.27
	Lower	517.06–1,305.20	865.10±12.55	186.10±17.75	
Buda C. I. 326	Upper	581.34–1,275.08	901.43±13.66	143.20±9.65	52.15±19.05
	Lower	597.38–1,104.40	849.28±13.28	138.60±9.35	
Susceptible:					
Winona C. I. 481	Upper	416.66–858.42	606.22±8.84	92.74±6.25	87.45±11.70
	Lower	366.46–677.70	518.77±7.70	78.05±5.27	
Indian Type 22	Upper	376.70–702.80	518.49±8.84	92.72±6.25	31.97±13.13
	Lower	301.20–778.10	486.52±9.71	102.00±6.88	
Deep Pink C. I. 648	Upper	351.40–798.19	519.76±9.74	102.00±6.88	89.85±11.86
	Lower	291.16–552.20	429.91±6.78	71.05±4.79	
Crepitans C. I. 506	Upper	281.12–677.70	463.85±7.36	77.01±5.12	35.04±9.93
	Lower	266.06–602.40	428.81±6.67	59.80±4.70	

A similar experiment was made to ascertain the pressure required to puncture the epidermis of stems of the same varieties and plants used in determining the pressure required to puncture the leaf epidermis. The results are given in table 5. It is evident that the stems of the susceptible varieties examined were far easier to puncture than those of the resistant and immune varieties.

TABLE 5.—*Pressure required to puncture the outer epidermal wall of stems of varieties of flax differing in their reaction to rust*

Reaction and variety	Range	Mean	Standard deviation
Immune:	*Milligrams*	*Milligrams*	*Milligrams*
Minnesota Selection C. I. 664	913.64–1,346.36	1,142.14±9.78	102.5 ±6.91
Minnesota Selection C. I. 661	978.90–1,286.12	1,148.40±7.50	78.8 ±4.37
Indian Type 46	993.96–1,321.26	1,153.03±7.90	82.8 ±5.59
Resistant:			
Indian Type 12	848.38–1,249.98	1,078.50±8.73	91.4 ±6.17
Lino Grande C. I. 381	908.62–1,301.18	1,090.86±8.77	90.9 ±6.13
Buda C. I. 326	828.30–1,104.00	954.54±8.70	91.1 ±6.14
Susceptible:			
Winona C. I. 481	702.80–1,109.42	872.76±10.00	104.8 ±7.07
Indian Type 22	652.60–933.72	804.04±6.64	69.71±6.64
Deep Pink C. I. 648	502.00–853.40	651.39±8.90	93.5 ±6.31
Crepitans C. I. 506	692.76–888.54	789.14±5.60	58.6 ±3.96

The results of all the foregoing determinations are summarized diagrammatically in figure 8, from which it is evident that there is a definite correlation, in the varieties examined, between rust reaction and the resistance of the epidermal membrane to puncture. The leaves of resistant varieties were found to be more resistant to puncture than those of immune varieties, while those of immune varieties were more resistant to puncture than the leaves of susceptible varieties, a mathematically significant difference existing in nearly every instance between the pressures required to puncture the upper and lower leaf surfaces. The stem epidermis of the resistant varieties examined was found to be more resistant to puncture than

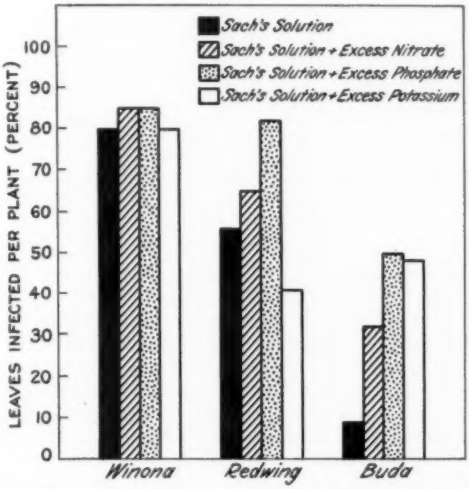

FIGURE 8.—Diagrammatic summary of the pressures required to puncture the leaf and stem epidermis of flax varieties differing widely in their reaction to flax rust, as determined by means of the modified Joly balance.

that of susceptible varieties, very little difference existing between resistant and immune varieties, although the latter required a slightly greater pressure to effect puncture.

The differences in the pressure required to puncture the leaf and stem epidermis of the different varieties of flax examined would indicate that the toughness of the epidermal membrane may play a role in determining or limiting formation of uredia in varieties of cultivated flax differing widely in their reactions to *Melampsora lini*.

Melander and Craigie (*34*), using the same methods as were employed in this investigation, obtained results indicating that those species of *Berberis* that possessed leaves very resistant to puncture were usually resistant to *Puccinia graminis* also. In addition, leaves of certain resistant species were found to have a very thick cuticle. Resistance of barberry leaves to puncture was found to increase with age of the plant, and this in turn was correlated with increased resistance to infection by stem rust.

Hawkins and Harvey (*18*), using a modified form of Joly balance, found that more pressure was required to puncture the tissues of a variety of potato comparatively resistant to leak, caused by *Pythium debaryanum* Hesse, than was required to puncture the tissues of two susceptible varieties. Correlated with this resistance to puncture is a resistance to infection by *P. debaryanum*.

Leach (*29*), studying the parasitism of *Colletotrichum lindemuthianum*, found that the inner cell wall of susceptible host tissues offers a certain amount of resistance to the growth of the mycelium of the anthracnose organism, retarding apical growth and causing the mycelium to become greatly enlarged and to bend outward before penetration of the cell wall is effected. In old tissues of susceptible varieties of beans, the mycelium is retarded in its development by the increased resistance of the cell walls to penetration. Such retarded mycelium disintegrated, killing the host protoplast, and staining the host cell wall and its contents reddish brown. Growth of the mycelium and penetration of cell walls was studied in sections of living material. The fungus was observed to penetrate through numerous cells without killing the protoplast of the host. The swelling and bending of the hyphae during cell-wall penetration would indicate that mechanical pressure is an important factor in early stages of development of *C. lindemuthianum* in the tissues of the bean.

An interesting analogy may be drawn between the results obtained in the present investigation and those cited above. In the case of the barberry, the presence of a thick cuticle and the resistance of the epidermal membrane to puncture offers an effective barrier to penetration by the sporidial germ tubes of *Puccinia graminis*. Thus, in this example, the resistance of the epidermal membrane prevents the pathogen from entering the host plant, and in certain cases is directly responsible for the resistance of some species of *Berberis* to infection.

In the case of potatoes resistant to *Pythium debaryanum* and beans resistant to *Colletotrichum lindemuthianum*, the degree of the resistance of the cell walls to penetration apparently determines the rate of development of the causal organisms after they have gained entrance to their respective hosts.

In the case of flax rust the toughness of the epidermal membrane in certain flax varieties prevents normal formation of uredia, as the force exerted by the growing uredium is apparently insufficient to rupture the covering epidermal membrane, resulting in the presence of subepidermal uredia and the failure of the urediospores to be liberated. Such a condition may be of considerable significance under field conditions. It is possible that the epidermis of these varieties may prevent normal penetration by sporidial germ tubes, but this phase of the problem was not studied.

OCCURRENCE AND ARRANGEMENT OF THE CORTICAL FIBERS

The occurrence and arrangement of the cortical fibers varies greatly in different flax varieties, and probably also under different environmental conditions. In some varieties they occur as irregular, thin-

walled, isolated, or grouped fibers not forming a continuous ring; in others they occur as regular, thick-walled fibers forming a continuous ring in the cortex of the stem. Cross sections of the cortical fibers in several varieties of flax are shown in figure 9.

It will be noticed that in the immune variety Rio (fig. 9, *E*) the cortical fibers are very thick-walled, somewhat square in cross section, and form a continuous band of cells in the cortex of the stem, whereas in another immune variety, Indian Type 46 (fig. 9, *A*), the fibers are very small and occur in isolated groups.

In the resistant variety Lino Grande (fig. 9, *F*), the fibers are small, thin-walled, irregular, and arranged in scattered groups 1

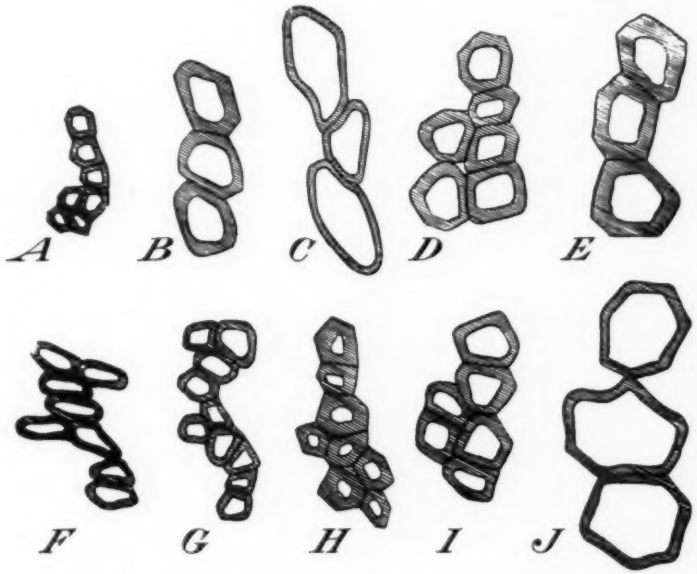

FIGURE 9.—Cortical fiber types in different varieties of flax: *A*, Indian Type 46; *B*, M–25–221; *C*, Deep Pink; *D*, Crepitans; *E*, Rio; *F*, Lino Grande; *G*, Bolley Golden; *H*, Pinnacle; *I*, Winona; *J*, Buda.

to 3 cells deep. In the variety M–25–221 (fig. 9, *B*), also resistant to rust, the fibers are fairly large, thick-walled, somewhat square in cross section, and arranged in a discontinuous ring in the cortex of the stem. In the resistant variety Bolley Golden, (fig. 9, *G*), the fibers are small, thin-walled, and occur in small groups scattered irregularly throughout the cortex. In the susceptible varieties there are extreme variations in the size, shape, and arrangement of the fibers. In Deep Pink and Buda they are large, rectangular in cross section, thin-walled, and scattered. In Winona, Crepitans, and Pinnacle (fig. 9, *I*, *D*, *H*), on the other hand, they are thick-walled, tend to be square in cross section, and form a more or less continuous band in the cortex. In these varieties the cortical fibers are par-

ticularly thick-walled and form a well-developed, continuous band of cells 1 to 3 cells deep.

From these observations it is obvious that the occurrence and arrangement of the cortical fibers in flax cannot be connected in any way with the morphological resistance of varieties to rust.

TO WHAT EXTENT DOES THE MORPHOLOGY OF THE HOST OPERATE IN THE RESISTANCE OF FLAX TO RUST?

From the results of the foregoing studies, it is apparent that there are no gross anatomical features comparable to the sclerenchyma girders in the peduncle of the wheat plant which might be a factor in determining the resistance or susceptibility of flax to rust. The fact that the leaf and stem epidermis of certain varieties resistant to rust in the field offers far more resistance to puncture than does the epidermis of certain susceptible varieties may be of considerable importance. Such varieties possessing an epidermal membrane capable of withstanding relatively high strains and thus rupturing only after considerable force has been exerted have been shown to be resistant to rust in the field. While most of these varieties usually produce the characteristic type 1 reaction, with the appearance of flecks and no urediospores on the leaves, a few small scattered uredia occasionally occur on the leaves of these varieties. If one examines such leaves, well-developed uredia containing numerous urediospores will often be observed immediately beneath the epidermis. The urediospores are pressed tightly against the epidermal cells; consequently they are much flattened at the ends and molded to the contours of the epidermal cells, indicating that they have been subjected to considerable pressure exerted by the stromatic mass upon which they were borne. Obviously, the urediospores produced under such conditions may never be liberated from the host and dispersed. Under field conditions this probably would result in a significant decrease in the amount of inoculum liberated in the course of the summer and a consequent reduction in the prevalence of rust.

Correlated with the resistance of the epidermal membrane to puncture is a shortening and thickening of the individual epidermal cells. In most of the varieties with epidermal membranes relatively resistant to puncture the epidermal cells were short and isodiametric, thus accounting, in part, for the amount of pressure required to effect puncture.

It must be admitted that the resistance of a membrane to puncture from without, as determined by the modified Joly balance, may not be a true indication of its resistance to rupture from within. Nevertheless, it is logical to suppose that such determinations do give an indication of the resistance of the epidermal membrane to rupture, and, in fact, the methods used in this work appear to be the only practical means of studying this question.

In addition to having membranes resistant to puncture, the epidermal cells of certain resistant and immune varieties had extremely thick cuticles. Furthermore, the stems of such varieties invariably were provided with a more or less continuous layer of hypodermis immediately below the epidermis.

The fact should not be overlooked that the presence of a thick epidermal membrane, the occurrence of a thick cuticle, and the presence of a hypodermis in some resistant and immune varieties may prevent penetration of the sporidial germ tubes of *Melampsora lini*, as is the case with *Berberis* spp. and *Puccinia graminis*. If this is true, such resistant and immune varieties would escape infection by sporidia of the rust fungus, provided the sporidial germ tubes were unable to penetrate the thick membrane; and, if infection subsequently did occur, it would have to be through the medium of urediospore germ tubes entering through the stomata. Such a condition would obviously result in a significant reduction in the amount of available inoculum in the early summer, and, in addition, the varieties which escaped sporidial infection would not be exposed to infection by urediospores until relatively mature, which might be a factor to be considered. This probability has not been investigated, but it appears to be worthy of careful consideration.

It is apparent that certain morphological features of the flax plant do operate in the resistance of some varieties to rust in the manner outlined above. While these factors alone may not be sufficient to confer resistance, they probably contribute to the resistance of some varieties.

RELATION OF STOMATA TO RUST RESISTANCE

In recent years the "functional" type of resistance has been suggested as a possible factor in resistance to certain diseases. According to the functional resistance hypothesis, the number, size, and movement of the stomata of the host plant are considered in certain cases to contribute toward its resistance to those diseases the causal organisms of which gain entrance to the suscept by way of the stomata. In the case of the rusts the urediospore is liberated, and if it eventually reaches a suitable infection court it proceeds to germinate, provided environmental conditions are favorable. The germ tube continues to grow until it reaches an open stoma, through which it sends an infection peg into the interior of the host, thus initiating infection. Recent investigations have shown that in cereals, and in fact in most plants, there is a regular diurnal movement of stomata, which varies under conditions of a fluctuating environment (*33*). In addition, stomatal movement in wheats has been found to vary in different varieties within the species. Such variations seem sometimes partially to determine the resistance or susceptibility of the variety to stem rust. Thus, the stomata of certain highly susceptible varieties, such as Little Club, Reward, and Quality, open almost immediately at sunrise and remain open during the hours of sunlight, thus allowing ample opportunity for the entrance of urediospore germ tubes. Certain highly resistant varieties, like Webster, Hope, and Velvet Don, on the other hand, do not open their stomata until the dew has disappeared from the leaves, and thus the urediospore germ tubes, in the absence of adequate moisture, fail to extend sufficiently to enter through a stoma when the stoma does open (*17*). Such varieties, by virtue of their stomatal movement, may escape

infection to some extent and are said to possess "functional resistance" to stem rust. It was thought possible that certain flax varieties might have a functional resistance to *Melampsora lini;* therefore, the following experiments were made.

PREVIOUS WORK

Pool and McKay (*40*), studying the relation of stomatal movement of *Beta vulgaris* to infection by *Cercospora beticola* Sacc., found that germ tubes penetrated sugar beet leaves only through open stomata. They found also that stomatal movement was influenced by leaf maturity and by certain environmental conditions. In mature leaves, stomatal movements were more active than in young heart leaves, while only very slight movements were observed in old leaves. The results of inoculations established the fact that heart and very young leaves were not susceptible to *C. beticola*, young mature leaves were only slightly so, and mature leaves were quite susceptible. At daily temperatures of 70° to 90° F., with a relative humidity of about 60 percent, the stomata on the mature leaves remained open throughout the day. In certain of the experiments very long germ tubes were produced, but in no case were they observed to penetrate through closed stomata, penetration taking place only through open stomata.

Caldwell and Stone (*7*), by a method of stripping the epidermis from wheat seedling leaves inoculated with *Puccinia triticina* Eriks. and by fixing and mounting the strips in absolute alcohol, were able to study directly the relation of stomatal aperture at the time of leaf penetration to the entrance of rust into the host. Closed stomata were found to offer no barrier to the penetration of wheat seedlings by urediospore germ tubes of the rust; the studies indicated rather that the formation of an appressorium over an open stoma stimulated it to close tightly prior to penetration by the rust. Occasionally a small stomatal slit was evident between the appressorium and the substomatal vesicle, apparently resulting from the penetration tube pushing between the guard cells of the stoma.

Hart (*17*) found that germ tubes of *Puccinia graminis tritici* enter wheat only through open stomata, for apparently the urediospore germ tubes are incapable of forcing their way between the guard cells when the stomata are closed. She concluded that the uredial stage of *P. graminis tritici* causes infection only when there is sufficient moisture for spore germination, when favorable temperatures for urediospore germination prevail, and while the stomata are sufficiently open to permit the entrance of the germ tubes. Allen (*1*), studying *P. triticina* on Little Club wheat, found that in material fixed in the early morning, germ tubes had just entered the host plant and formed substomatal vesicles when examined histologically. On material fixed in the afternoon most of the substomatal vesicles had sent out infection hyphae. She suggests that the time of entrance of *P. triticina* may be conditioned by the daily stomatal movements of the host and that entrance depends upon the natural opening of the stomata rather than upon mechanical force or chemical action.

Hart (*17*), from extensive investigations of the relation of stomatal movements in wheat to resistance to stem rust, concluded: "Certain wheat varieties may escape stem-rust infection because of their characteristic stomatal behavior. Such varieties are said to possess functional resistance to stem rust under certain conditions."

NUMBER OF STOMATA ON LEAVES OF DIFFERENT VARIETIES OF FLAX

Since the urediospore germ tubes of *Melampsora lini* enter the host through stomata, it would be reasonable to suppose that those varieties with the greatest number of stomata per unit area of leaf surface might be the most susceptible to rust, other factors being the same. It therefore seemed desirable to determine the number of stomata on the leaves of flax varieties that differed widely in their reaction to rust.

Several varieties were grown in the greenhouse and the number of stomata on seedling and mature leaves determined. The leaves were removed from the plants and placed immediately in acetoalcohol until clear and then stained in lactophenol, cotton-blue stain. Using the lower power objective of the microscope, the number of stomata on the upper and lower leaf surfaces within a leaf area of 0.5 mm^2 was determined by placing within the ocular of the microscope a cover slip bearing a measured area corresponding to 0.5 mm^2 of leaf surface and counting the number of stomata within that area. The number of stomata within a definite leaf area was determined for the apex, middle, and base of the upper and lower leaf surfaces of each leaf. Several leaves of each variety were used, and 10 microscopic fields were examined for each of the above-mentioned portions of the leaf. The figures given in table 6 thus each represent the average of 10 determinations, the average number of stomata for both surfaces being the average of 60 determinations. The rust reactions of the varieties were recorded during the summer of 1932 in the flax rust nursery at Coon Creek. From the results given in table 6 it will be noticed that the seedling leaves of varieties susceptible to *Melampsora lini* consistently had a larger number of stomata per square millimeter of leaf surface than those of immune varieties. The differences appear to be greater than those between susceptible and resistant varieties. No significant differences are apparent in the number on the upper and lower leaf surface. In some susceptible varieties the apex of the seedling leaves appears to have a greater number of stomata than the middle or basal portions. It seems significant that field observations indicate that infection and subsequent uredium formation appear first at the apex of the leaves. The differences in stomatal number of the various portions of the leaves are more marked in mature leaves (table 6), and the presence of a greater number of stomata at the apex might account for the appearance of the first-formed uredia at the apices. Comparable results were obtained when the stomatal number for mature leaves was determined (table 6). The leaves of susceptible varieties again possessed a slightly greater number of stomata than the immune varieties, the difference between susceptible and resistant varieties being less marked.

TABLE 6.—*Number of stomata on seedling and on manure leaves of varieties of flax differing in their reaction to rust*

(Each figure representing the average of 10 microscopic fields, each containing an area equivalent to 0. mm² of leaf surface)

SEEDLING LEAVES

Reaction, variety, and surface	Average leaf dimensions		Average stomata in 0.5 mm² of leaf surface					Average stomata per square millimeter of leaf surface based on average for both surfaces	Rust reaction	
	Width	Length	Apex	Middle	Base	Average	Average both surfaces		Prevalence	Type no.
Immune:	*Centimeters*	*Centimeters*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*	
Minnesota Selection C. I. 651	0.76	1.58					13	26	0	
Upper			14	14	12	13				
Lower			13	13	12	13				
Minnesota Selection C. I. 661	.75	1.56					16	32	0	0
Upper			17	16	16	16				
Lower			17	16	16	16				
Indian Type 46	.66	1.41					18	36	0	0
Upper			18	18	18	18				
Lower			17	17	18	17				
Resistant:										
Indian Type 68	.91	1.77					20	40	5	1
Upper			21	20	18	20				
Lower			20	21	18	20				
Bolley Golden C. I. 644	.78	1.42					19	38	50	1
Upper			19	20	17	19				
Lower			20	20	17	19				
Redwing C. I. 320	.75	1.36					19	38	15	1
Upper			20	20	18	19				
Lower			19	19	18	19				
Susceptible:										
Bison C. I. 389	.96	1.67					20	40	1–15	3–4
Upper			20	20	18	19				
Lower			20	20	19	20				
Pinnacle C. I. 693	.66	1.23					22	44	60	4
Upper			24	22	20	22				
Lower			24	23	20	22				
Crepitans C. I. 506	.70	1.59					22	44	70	4
Upper			24	23	20	22				
Lower			23	24	17	21				

MATURE LEAVES

Immune:										
Minnesota Selection C. I. 651	0.33	1.51					24	48		
Upper			25	25	25	25				
Lower			25	23	22	23				
Minnesota Selection C. I. 661	.42	1.67					24	48		
Upper			24	24	24	24				
Lower			25	23	22	23				
Indian Type 46	.34	1.61					23	46		
Upper			26	24	22	24				
Lower			23	22	21	22				
Resistant:										
Bolley Golden C. I. 644	.44	1.84					28	56		
Upper			30	28	28	29				
Lower			29	26	26	27				
Redwing C. I. 320	.41	1.67					26	52		
Upper			28	26	26	27				
Lower			28	26	25	26				
Bison C. I. 389	.48	1.92					25	50		
Upper			26	25	25	25				
Lower			24	25	23	24				
Susceptible:										
Pinnacle C. I. 693	.39	1.48					28	56		
Upper			29	27	25	27				
Lower			32	26	25	28				
Deep Pink C. I. 648	.40	1.66					28	56		
Upper			30	29	28	29				
Lower			26	27	27	27				

Hursh (*22*), from a study of the nature of resistance of wheat to *Puccinia graminis tritici*, concluded that there was no relation between the stomatal number in wheats and their degree of resistance to stem rust. In the present experiments, although differences were observed in the number of stomata on leaves of different varieties of flax, they do not appear to be sufficiently significant alone to determine the resistance of a specific variety to rust. However, it should be pointed out that the differences in stomatal number of the varieties recorded are based on the number of stomata per square millimeter of leaf surface. If the total leaf area of plants of different flax varieties were considered, the differences in the total number of stomata on a plant such as the variety Deep Pink, and the total number on a plant of the variety Indian Type 46, might be quite significant. Although the presence of a greater number of stomata on the leaves of a certain variety might not necessarily render it more susceptible to rust than a variety with fewer stomata per leaf, such differences may possibly be of significance when correlated with other factors, such as stomatal movement. Furthermore, the presence of a greater number of stomata on the leaves of a susceptible variety may not be of importance when conditions for rust infection are at their optimum, but under conditions of low light intensity, low humidity, and scarcity of urediospores, it may be of considerable importance in determining the degree of infection on these leaves.

STOMATAL MOVEMENTS IN RELATION TO RESISTANCE

Several methods of observing changes in stomatal apertures were carefully considered, but owing to the small size of the stomata of the flax leaf most of these methods proved impractical. Thus, the cobalt-paper method of Trelease and Livingston (*43*), the various porometer methods of Darwin and Pertz (*9*), Knight (*24*, *25*), and Laidlaw and Knight (*27*) are entirely unsuitable. The method of direct microscopical observation of the stomatal apertures of plants growing in the field, devised by Lloyd (*32*), is of limited practicability with flax, the small size of the stomatal aperture making it very difficult to distinguish between fully open and partially open stomata. The method of stripping off the epidermis from the leaf and quickly plunging it into absolute alcohol, also devised by Lloyd (*31*), was found to be the most practical for this investigation. The effect of absolute alcohol on strips of leaf epidermis is to dehydrate the cell wall and cause it to become stiff and hard before removing the water from the cell. As a result, the cell walls retain their original shape and the size of the stomata remains unaltered as long as the material is kept dehydrated. The reliability of this method has been verified by Loftfield (*33*), who compared the dimensions of the stomata of leaves in position with those of the stripped epidermis treated with absolute alcohol and found that the measurements were identical. On the strength of this evidence the method was adopted for the present work.

Strips of leaf epidermis were removed from several varieties of flax growing in the field, plunged immediately into absolute alcohol, and then stored in small vials to be examined microscopically in the laboratory later. Epidermal strips were removed from several ma-

ture leaves from different plants every hour during the course of the experiments. Temperature and relative humidity records were kept, together with observations on the hour of sunrise, intensity of light, and the duration of dew. The epidermal strips were examined microscopically in the laboratory, and camera lucida drawings were made of several typical stomata in each series. A typical stoma was then selected from this collection of tracings and transposed to a chart designed to depict diagrammatically and to compare the condition of the stomatal apertures of different flax varieties at different

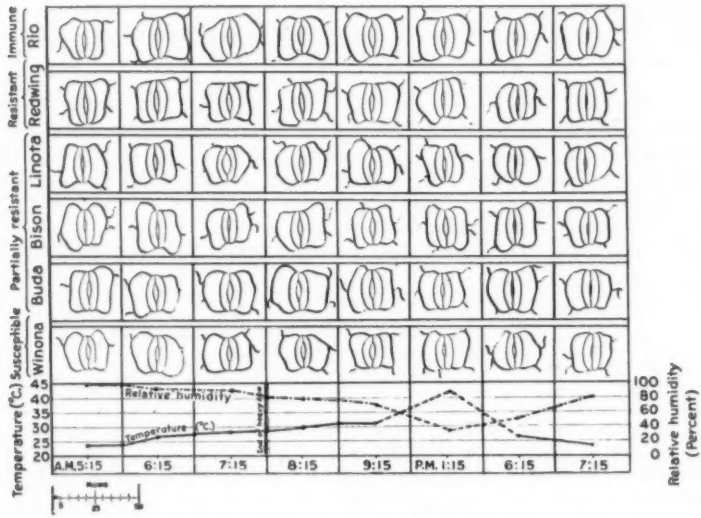

FIGURE 10.—Diagrammatic representation of the stomatal movement of different flax varieties under normal field conditions of light, temperature, and humidity at a time when conditions for infection were optimum. (Camera lucida tracings.)

periods of time. Observations were made from June 30, when conditions for natural infection by urediospores of rust were considered optimum.

As a preliminary experiment epidermal strips were removed from mature leaves of Winona, Buda, Bison, Redwing, and Rio flax plants in the preflowering stage, growing in the experimental plots at University Farm. The epidermal strips were handled in the manner outlined above, and a summary of the tracings obtained from these preparations is given in figure 10. It will be noticed that the stomata of the susceptible variety Winona were fully open at sunrise and remained so until noon, when they began to close, being reduced to narrow slits by late evening. In the partially resistant varieties, Buda, and Linota, the stomata were just beginning to open at sunrise, and attained their greatest aperture at about 7:15 a. m.; then they began to close, finally becoming reduced to narrow slits by 7:15 p. m. In the case of Bison, which is partially resistant to rust, the stomata opened rather slowly, and in this experiment did not become fully open until after the dew had evaporated, namely, at 9:15 a. m.

In the resistant variety Redwing the sequence was similar to that in the three previously mentioned varieties, the stomata opening rather slowly, becoming wide open at about 7:15 a. m., and commencing to close at 1:15 p. m. In the immune variety Rio, the stomata opened comparatively slowly, attaining their greatest aperture at 9:15 a. m., after the dew had disappeared.

From this experiment it would seem that at no time while the dew was on the leaves and while conditions were at their optimum for infection were the stomata of any variety closed sufficiently to prevent the entrance of the urediospore germ tubes. The stomata of all varieties, with the possible exception of Bison, had opened to approximately half their maximum aperture by the hour of sunrise, and this would not seem to interpose an effective barrier to germ-tube penetration.

At this point another experiment was made to determine whether there was any difference in the stomatal movement of the upper and lower leaf surfaces of the flax plants. Using the same technic as in the previous experiment, the strips of leaf epidermis were removed at hourly periods from the upper and lower leaf surfaces of Bison and Rio flax plants growing in the experimental field plots. Camera lucida tracings of the stomata of these samples were made and representative specimens are shown in figure 11, *A*. From a study of this chart, it is apparent that there is relatively little difference between the stomatal movements of the upper and lower leaf surfaces. The stomata of the lower leaf surface do appear to lag behind those of the upper surface slightly and perhaps do not open as wide, doubtless because the sun's rays hit the upper leaf surface first, and the amount of light received by the upper surface must be more than that which reaches the lower leaf surface, because of the manner of attachment of the leaf to the stem.

In a previous experiment the stomatal movements of several flax varieties were studied under conditions of bright sunlight and heavy dew. It was thought desirable to study the stomatal movements under the other extreme of conditions. Accordingly, the stomatal movements of the upper epidermis of Winona, Bison, Pale Blue, and Redwing flax were studied on a cloudy day when there was no visible dew and the light intensity was low. The movements of the stomata on the upper epidermis of the four varieties mentioned are illustrated in Figure 11, *B*. Again it will be noticed that the stomata of all of the varieties were approximately half open at sunrise, and a comparison with figure 10 would indicate that low light intensity and low humidity did not materially influence stomatal movement in this instance.

EFFECT OF DARKNESS DURING THE INCUBATION PERIOD ON RUST DEVELOPMENT

Although there are indications that stomatal movement in certain varieties may operate in resistance, it is not possible, on the basis of the foregoing experiments, to state definitely what effect stomatal movements may have in determining the resistance of certain varieties to rust. Since the methods used in these experiments were rather crude and decidedly unnatural it was considered advisable to approach the problem in a different manner.

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Accordingly, seeds of Winona, Redwing, and Buda were planted in the greenhouse, and when the seedlings had attained a height of approximately 8 inches, one series was placed in an incubation cham-

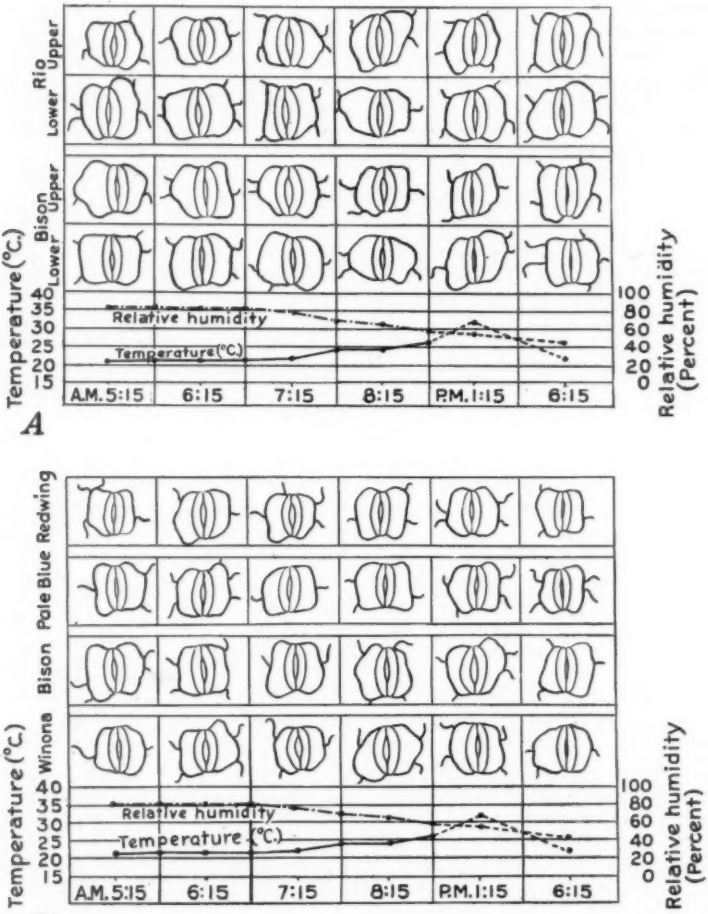

FIGURE 11.—Diagrammatic representation of the stomatal movements of different flax varieties under field conditions. (Camera lucida tracings.) *A*, Comparison of the stomatal movement of the upper and lower leaf surfaces of Bison and Rio flax; *B*, stomatal movements of the upper leaf surface of Winona, Bison, Pale Blue, and Redwing flax under conditions of low humidity and low light intensity.

ber from which all light was excluded by means of sheets of heavy paper, and a second series of the same varieties in the same stage of maturity was placed in another chamber from which the light was not excluded. The plants were left in these chambers for 24 hours and were then uniformly inoculated. The inoculated plants

were then incubated for an additional 48 hours. Both series were kept side by side in the greenhouse during this period. The object of this procedure was to determine whether closing of the stomata by the exclusion of light would influence the development of rust. Thus the plants in each series were incubated for 24 hours prior to inoculation and 48 hours subsequent to inoculation, one series being in total darkness during the entire 72 hours, the other being in normal diffused light for the same period. Forty-eight hours after inoculation, the plants were removed from the incubation chambers and placed side by side in the greenhouse. The plants were carefully examined for differences in vigor, but no differences were observed. No visible symptoms of etiolation were apparent on plants of the series incubated in the dark. Ten days after inoculation the total number of leaves on each plant and the number of infected leaves on the same plant were counted for each variety in both series. The results given in table 7 are therefore the average of approximately 25 plants in every case. The number of uredia on individual leaves of different plants also were counted; the figures for the average number of uredia per leaf given in table 7 represent the average of approximately 25 leaves in each case. In determining the percentage of leaves infected, only green leaves susceptible to infection at the time of inoculation were considered, the rosette of very young leaves at the apex of each plant being excluded when the counts were made.

TABLE 7.—*Effect of darkness during the incubation period on rust development on three flax varieties*

Variety	Rust reaction	Incubated in dark				Incubated in light			
		Total leaves per plant	Leaves infected per plant		Average pustules per leaf	Total leaves per plant	Leaves infected per plant		Average pustules per leaf
		Number	*Number*	*Percent*	*Number*	*Number*	*Number*	*Percent*	*Number*
Winona	Susceptible	16	11	69	5	19	16	84	9
Redwing	Partly resistant	38	12	32	2	59	47	80	7
Buda	Resistant	53	3	6	1	44	8	18	2

From the results presented in table 7 it is apparent that darkness during the incubation period resulted in a significant reduction in the percentage of rust-infected leaves per plant and also in the number of uredia developed per leaf, the series incubated in the dark having consistently less rust than that incubated in normal light. Furthermore, darkness during the incubation period influenced rust development on normally resistant varieties to a much greater extent than it did on the susceptible variety Winona. Hart (*16*) found that darkness does not influence the germination of urediospores of *Melampsora lini* to any extent, and observations by the writer corroborate her findings. The temperature in the dark incubation chamber was about 3° lower than in the chamber from which the light was not excluded (viz. 21° C.). This temperature difference does not seem great enough to explain the fore-

going results, especially the difference in number of uredia. The humidity in the two chambers appeared to be approximately the same, or at least was adequate for optimum infection, and no differences in the succulence of the plants in the two series were apparent after incubation. Germination studies showed that the germ tubes of the rust organism were not heliotropic as they ramified in all directions irrespective of the source of light. Directing the source of light so as to form a beam failed to result in a response in the direction of germ-tube growth. Particular pains were also taken to ensure uniform inoculation of all the plants in both series.

The elimination of the foregoing factors seems to indicate rather strongly that the closing of the stomata of plants incubated in darkness following inoculation with rust may possibly explain why such plants were not so heavily infected as when they were incubated in normal diffused light. These experiments were repeated with similar results. It would be desirable, however, to check carefully the above findings, using additional varieties and plants in various stages of maturity. In addition, attempts should be made by means of microscopical observation of inoculated leaves in surface view, to determine the actual percentage of germ tubes entering the plants, and the percentage of germ-tube entrance through the stomata should be compared on plants incubated in darkness and in normal diffused light.

EFFECT OF FLAX WILT ON STOMATAL MOVEMENT IN RELATION TO RUST DEVELOPMENT

For a number of years certain experimental plots at University Farm have been used for a study of flax wilt, caused by *Fusarium lini* Bolley, and as a result the soil has become thoroughly infested with inoculum. It is rather a striking fact that flax rust has not, up to the present time, been noticed on the flax varieties growing in these wilt-infested plots. The nonoccurrence of flax rust in these plots may have been due to a number of causes. (1) The humidity in this locality at the time when infection is likely to occur may have been too low to permit successful infection. This seems unlikely, as leaf and stem rust of wheat have been very prevalent in the immediate vicinity. (2) It may have been due to an absence of sufficient inoculum, which does not seem probable, as epidemics of flax rust have occurred during the past 3 years, at least on plants growing within a distance of 1 mile, and one would expect that sufficient urediospores would be blown from these infected plants to cause the appearance of at least some rust in the flax-wilt plots. (3) The failure of rust to appear in these plots may have been due to certain soil factors which were unfavorable for the development of rust, and which might also have resulted in the failure of the stomata to open, thus preventing the entrance of urediospore germ tubes of flax rust. The plants in these plots are planted in short, widely separated rows, which would probably reduce the rate of spread of the disease but certainly would not prevent its appearance.

On June 27 the stomatal movements of a number of flax varieties in the early-blossom stage in wilt-sick soil were examined at hourly intervals, using the technic previously described. Observations were

made on the following varieties: Buda, Bison, Pale Blue, Winona, Redwing, Linota, and Pale Pink. The plants, although growing in wilt-sick soil, at this time had no symptoms of wilt and appeared to be healthy and turgid. When epidermal strips were removed from the plants and examined under the microscope, it was found that, in most varieties, stomata were completely closed at all times, while in the remaining varieties they apparently had never opened to more than a narrow slit. At first it was thought that the technic used in removing and fixing the epidermal strips might have been faulty, causing the collapse of the guard cells and the subsequent closing of the stomata. The experiment was therefore repeated, dehydrating the epidermal strips in fresh absolute alcohol, but the results were the same. These observations were repeated several times and checked with observations of the stomata of flax plants growing in healthy soil. In all cases the stomata of the plants growing in the wilt-sick soil apparently failed to open, whereas those of plants growing on healthy soil and examined at the same time opened in the normal way.

It would thus appear possible that the presence of *Fusarium lini* in the soil resulted in the death of the guard cells and the subsequent nonfunctioning of the stomata of the plants concerned.

Iljin (*23*), in 1922, reported that in certain plants which were suffering from physiological wilt, the guard cells invariably died and the stomata ceased to function.

In the light of the above observations it would seem possible that the failure of flax rust to appear in the wilt-sick plots was due to the fact that the stomata of the plants growing in this soil for some reason failed to open and thus prevented the germ tubes of flax rust from entering. The nonfunctioning of the stomata of these plants may in turn have been due to the effect of flax wilt on the physiology of the host plant.

TO WHAT EXTENT IS FLAX RUST RESISTANCE INFLUENCED BY NUMBER AND MOVEMENTS OF STOMATA?

In the preceding investigation of the stomata of the flax plant indications were obtained that certain immune and resistant varieties have fewer stomata per square millimeter of leaf surface than certain other varieties susceptible to rust. If one takes into consideration the total leaf surface of such plants it is possible that the differences in number of stomata on resistant and susceptible varieties may be of significance in germ-tube penetration in the former varieties, inasmuch as the germ tubes of urediospores enter the plant through the stomata.

Observations of stomatal movements of a number of flax varieties did not uncover striking differences in the stomatal movements of varieties differing in their reaction to rust. However, there were indications of slight differences in the rate of opening of the stomata of certain varieties. Such differences might well be a factor to be considered in view of the fact that the time during which germ-tube penetration can take place is of relatively short duration, being confined to the period when the dew is still on the leaves and while the stomata are sufficiently open to allow the entrance of the uredospore germ tubes, a period of approximately 3 to 4 hours, except,

of course, in case of rains. In a susceptible variety such as Winona the stomata have already attained their maximum width by the time the sun rises, and the opportunities for germ-tube entrance are favorable from the hour of sunrise, or from the time of stomatal opening, until the dew disappears from the leaves. In a variety such as Linota, on the other hand, the stomata do not attain their maximum aperture until 1 to 2 hours after sunrise and then remain fully open for 1 hour only. Thus the opportunity for germ-tube entrance by way of the stomata is confined to the brief period of 1 hour. It would seem that the chances of the germ tubes being able to grow sufficiently to enter a stoma during this period are comparatively slight. In the variety Bison the stomata do not open to their maximum width until the dew has disappeared from the leaves, and at this time conditions for germ-tube growth are decidedly unfavorable because of an inadequate film of moisture on the leaves. In the field Bison has been found to be partially resistant to rust, having the reaction 15 percent type 3 to 4, which means that approximately 15 percent of the total leaf surface was occupied by type 1 uredia. It is reasonable to suppose that the failure of the stomata of Buda to open until after the dew had evaporated may explain the low percentage of rust which normally develops on this variety in the field. It has not been determined whether urediospore germ tubes of *Melampsora lini* can penetrate closed or partially closed stomata, but it does seem entirely reasonable to suggest that partial closing of the stomata would offer at least some barrier to entrance of germ tubes.

When inoculated plants were incubated in darkness, there was decidedly less rust than on plants incubated in the light. By a process of elimination of several possible explanations for the above phenomenon it appears that the closing of the stomata on plants incubated in the dark may have been responsible. Here, apparently, is another indication that stomata of the flax plant may operate in influencing rust development. An examination of stomata of flax plants growing in wilt-infested soil revealed the fact that they were apparently closed at all times during the period of observation throughout the day. For a number of years rust has failed to develop in these plots, and the suggestion is advanced that the atrophy of the stomatal guard cells and the consequent closing of the stomata, possibly due to the presence of *Fusarium lini* in the soil, may have been responsible for the failure of rust to appear in these plots.

From a careful consideration of the above facts, one is forced to conclude that the stomata of the flax plant may, under certain conditions and with certain varieties, play a role in rust resistance. However, it should be pointed out again that differences in stomatal movements do not alone account for the susceptibility or resistance of varieties to rust.

It seems extremely important, when considering the role of stomata in plant disease resistance, to bear in mind the probability that under conditions optimum for infection, that is, when adequate moisture is present, when there is an abundance of inoculum, and when light conditions are favorable for normal stomatal movement, stomata may not be of much importance as determiners of the

resistance or susceptibility of a specific variety; however, when optimum conditions for infection do not prevail, that is, when dew and rain are absent, when urediospores are not abundant, and when low light intensities prevail, then stomata may be of considerable importance in influencing the amount of infection that is likely to occur on certain varieties.

EFFECT OF NUTRIENT SALTS ON RUST DEVELOPMENT

It is a commonly observed fact that the succulence and vigor of the host plant profoundly affect the susceptibility of the plant to rust, the more succulent and vigorous plants in general being more susceptible to rust. Hursh (*22*), working on the nature of resistance of wheats to stem rust (*Puccinia graminis tritici*), found that plants heavily fertilized with nitrogen may be more severely injured by stem rust than those not so fertilized. Heavy nitrogen fertilization was found to decrease the amount of sclerenchyma in proportion to the amount of parenchyma present in the wheat stem. Hart (*17*), contrary to the results obtained by Hursh, concluded from her experiments that there were no consistent or significant differences in the proportion or distribution of collenchyma in the peduncles of wheats grown in different fertilizer plots.

In this work attempt was made to determine whether the application of mineral salts would affect the development of rust on normally resistant flax varieties. Seeds of Winona, Redwing, Buda, and Rio flax were planted in 6-inch plots in sand, and until the time of emergence of the seedlings, were watered in the usual manner. Subsequent to the time of emergence, the plants were watered with the following nutrient solutions containing the indicated quantities of salts per 1,000 cc. of distilled water:

a. Sach's solution:	*Grams*
Potassium nitrate	0.4
Calcium sulphate	.5
Calcium phosphate	.5
Sodium chloride	.5
Magnesium sulphate	.5
Calcium carbonate	.5
b. Sach's solution plus excess nitrate:	
Potassium nitrate	1.2
Calcium sulphate	.5
Calcium phosphate	.5
Sodium chloride	.5
Magnesium sulphate	.5
Calcium carbonate	.5
c. Sach's solution plus excess phosphate:	*Grams*
Potassium nitrate	0.4
Calcium sulphate	.5
Calcium phosphate	1.5
Sodium chloride	.5
Magnesium sulphate	.5
Calcium carbonate	.5
d. Sach's solution plus excess potassium:	
Potassium nitrate	.4
Calcium sulphate	.5
Calcium phosphate	.5
Sodium chloride	.5
Magnesium sulphate	.5
Calcium carbonate	.5
Potassium sulphate	.5

Duplicate pots of each variety were used for the four series, and the nutrient solutions were applied by means of a pipette at the rate of 50 cc. per pot per day. Precautions were taken to ensure uniform stands of each variety in all pots.

Three weeks after planting, notes were taken on the height and succulence of the plants of each variety watered with the different nutrient solutions. The plants were then inoculated with fresh uredospore material, care being taken to inoculate each pot uniformly,

and they were then incubated in the usual way for 48 hours. Ten days after inoculation, notes were taken on the relative development of rust on the different varieties in each series. Counts were made of the total number of leaves per plant, the number of infected leaves per plant, and the number of uredia per leaf. The results, given in table 8, are thus the average for both pots of each variety, and the average number of uredia per leaf are the averages of approximately 25 leaves in each case.

TABLE 8.—*Effect of mineral salts on rust development in three flax varieties*

Variety	Nutrient solution	Average height of plants	Total leaves per plant	Infected leaves per plant		Average uredia per leaf
		Centimeters	*Number*	*Number*	*Percent*	*Number*
Winona	a. Sach's solution	8.8	40	32	80	16
	b. a+excess nitrate	11.6	40	34	85	14
	c. a+excess phosphate	10.6	40	34	85	20
	d. a+excess potassium	9.2	40	32	80	9
Redwing	a. Sach's solution	9.2	36	20	56	7
	b. a+excess nitrate	9.0	37	24	65	10
	c. a+excess phosphate	10.5	39	32	82	14
	d. a+excess potassium	8.2	34	14	41	4
Buda	a. Sach's solution	9.3	43	4	9	2
	b. a+excess nitrate	11.5	41	13	32	10
	c. a+excess phosphate	12.5	44	22	50	16
	d. a+excess potassium	9.5	42	20	48	7

The different nutrient solutions were found to influence the succulence and height of the plants to a noticeable degree. Excess phosphate especially seems to favor the growth of flax, the plants in the phosphate series being much more succulent and more vigorous than those watered with ordinary Sach's solution, and with Sach's solution plus excess potassium. The plants watered with the last two nutrient solutions were poorly developed and far less succulent than those watered with the nutrient solution containing excess phosphate and the solution containing excess nitrate. Excess potassium appeared to stimulate the growth of the plants very slightly, with the exception of Redwing, but the plants in this series were also poorly developed and less succulent than plants in the excess phosphate series. Excess nitrogen stimulated growth, and, in addition, the plants so treated were very vigorous and quite succulent. There appeared to be a differential response of certain of the varieties to the different nutrients, excess nitrate being most favorable for the growth of Winona, whereas excess phosphate was most favorable for the growth of Redwing and Buda. The effects of the different nutrient solutions on rust development are shown diagrammatically in figure 12.

It will be noticed from figure 12 that the application of excess phosphate favored rust development in all varieties. Excess nitrogen also stimulated rust development over the check series (ordinary Sach's solution), and excess potassium reduced the amount of rust development in Winona and Redwing, but not in Buda. In the case of Buda, excess potassium stimulated the growth of the plants slightly, which may account for more rust development on the Buda plants treated with excess potassium than on plants of the same variety treated with ordinary Sach's solution.

The interesting point in these results is the fact that the various nutrient solutions did not significantly alter the rust development on the normally susceptible variety Winona, but the application of excess phosphate did appear to influence significantly the susceptibility of the normally resistant varieties Redwing and Buda. Phosphate and nitrate application also increased the number of uredia produced on these two varieties. Increased susceptibility to rust of plants watered with excess phosphate and with excess nitrate is correlated with increased growth and succulence of the plants concerned. The

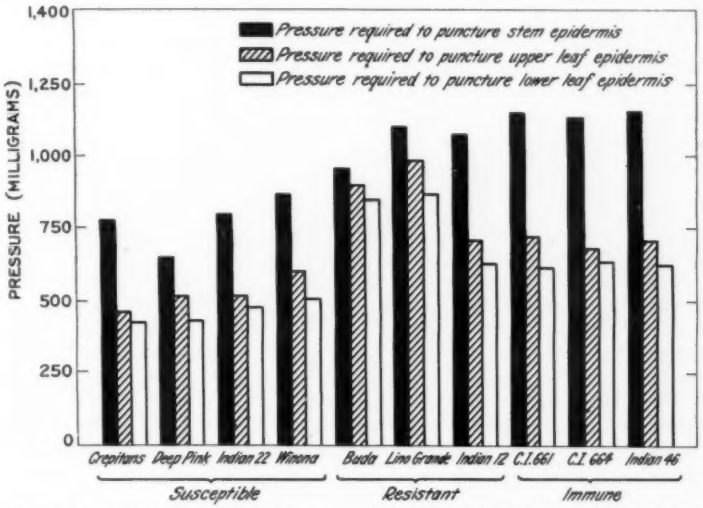

FIGURE 12.—Effect of nutrient salts on rust development in three varieties of flax.

different nutrient solutions failed to render susceptible the immune variety Rio.

From these results it appears that the application of excess nitrate and excess phosphate increased the development of rust, and excess potassium reduced it in the varieties studied.

DISCUSSION AND CONCLUSIONS

It is obvious that the resistance of cultivated flax to flax rust, *Melampsora lini*, is an extremely involved problem. The histological work demonstrated that certain varieties of flax exerted no protoplasmic antagonism toward the rust fungus, allowing the latter to develop extensively and to culminate its activities by the production of the overwintering teliospores, without encountering any apparent nutritional difficulties. Certain other varieties, on the other hand, appeared to erect nutritional barriers to the development of the rust fungus, restricting its development to a certain degree, but yet allowing it to complete its life cycle by the production of spores. Although the spores produced may be relatively few in number, they do nevertheless suffice to ensure the perpetuation of the species if suitable infection courts exist. Such varieties are

obviously resistant to rust, and the incompatability of host and parasite is evidenced by the necrosis of the host tissues.

In other varieties the protoplasmic antagonism of the host toward the fungus constitutes a barrier that the pathogen apparently cannot surmount, restricting its activities to the development of a sparse hyphal system and entirely preventing it from ensuring its perpetuation by the production of urediospores. The incompatibility of host and parasite in these varieties is evidenced by the complete necrosis of the host tissues in the infection zone, and by the failure of the rust to produce urediospores. Such varieties are considered to be highly resistant to flax rust.

In flax varieties immune to attacks by the rust fungus, the incompatibility between host and parasite is so complete that the latter fails to develop sufficiently to make its presence externally evident, even when it has gained entrance to the interior of the host.

Plant extracts obtained from varieties susceptible, resistant, and immune to rust were found to support the growth of urediospore germ tubes of the rust organism in precise agreement with the rust reactions of the varieties from which they were obtained. Thus it is obvious that the protoplasmic properties of the different flax varieties influence to a marked degree the ability of the rust fungus to establish itself and complete its normal cycle of development. In certain cases such properties may operate alone in determining the immunity of certain varieties; in other cases they apparently operate to a more limited extent and are not alone sufficient to prevent the development of rust. It must therefore be admitted that physiological properties of the host do operate in determining the resistance or susceptibility of a variety to rust, and further investigations of a physiological or serological nature would be desirable.

With regard to the role of morphological characters in resistance, it has been shown that there do not appear to be any gross anatomical features of the flax plant that can be considered as determiners of resistance. It has been demonstrated that the epidermis of varieties resistant to or immune from rust, without exception in the varieties studied, was far more resistant to puncture, as determined by a modified Joly balance, than was the epidermis of some susceptible varieties. Such a resistance to puncture was found to be correlated with the presence of a cuticle, isodiametric epidermal cells, and the development of hypodermal layers. It has previously been pointed out that such an epidermis, resistant to puncture, may operate in one of two ways, or in both, in influencing the resistance of a specific variety to rust. It has been suggested that an epidermal membrane resistant to puncture may prevent the entrance of sporidial germ tubes of the fungus just as that of certain immune varieties of barberry prevents entrance of those of *Puccinia graminis tritici*. Also, it has been shown that certain varieties possessing an epidermal membrane capable of withstanding relatively high tensile strains quite commonly develop subepidermal uredia and fail to liberate many of their urediospores. It is suggested that this fact would significantly reduce the amount of available inoculum pro-

duced in the course of the summer, provided these varieties were grown to any extent, and it may therefore be of considerable practical importance.

Thus it has been shown that morphological features of the host plant in certain cases play a part in the resistance of certain varieties to rust. While such factors do not seem to operate alone, it must be conceded that they contribute their share to the resistance of some flax varieties to rust.

Although no wide differences in the stomatal movements of different flax varieties were observed, indications were obtained that the rate of opening of the stomata may explain the low percentage of rust development on certain varieties. The fact that inoculated plants incubated in the dark developed less rust than similar plants incubated in the light would seem to support the opinion that the stomata of flax may play a part in resistance to rust. The fact that varieties of flax growing in soil infested with *Fusarium lini* have failed to rust for a number of years is interesting and significant in view of the observation that inoculum is abundant in this locality and conditions for infection are normally favorable. The observations of the stomata of these plants revealed the remarkable fact that they were apparently functionless because of certain soil factors or because of the presence of *F. lini* in the soil. It is suggested that this apparent nonfunctioning of the stomata may possibly explain the failure of rust to appear on plants growing in these plots. Again, this would seem to be additional evidence for the role of stomata as "cooperators" in influencing susceptibility to rust. Thus it must again be conceded that there is evidence that the stomata of the flax plant may contribute towards rust resistance.

In this investigation it was repeatedly observed that certain varieties resistant to rust in the field may be susceptible under greenhouse conditions. It has been shown that the application of certain nutrient salts, e. g., nitrate and phosphate, will favor the development of rust on varieties normally resistant under field conditions. In addition, plants in early stages of maturity appear to be more susceptible to rust than fairly mature individuals. This would suggest that environmental conditions and the general state of vigor of the plants undoubtedly influence the reactions to rust, and an investigation of a physiological nature along these lines would be desirable.

It has been shown that morphology, physiology, and stomatal movements of the flax plant may, under certain conditions and in certain varieties, all contribute their share to the resistance of a specific variety to rust. Certain of these factors may be of minor importance when optimum conditions for the development of rust prevail, but one should give very careful consideration to the possibility that they may be of great importance when optimum conditions for rust development do not prevail.

The resistance of cultivated flax to *Melampsora lini* cannot be attributed to any one single factor operating alone but must be considered to be due to a number of factors operating together to produce by their united efforts the condition termed "flax rust resistance."

SUMMARY

Flax rust, caused by *Melampsora lini* (Pers.) Lév., may be considered to occur wherever cultivated flax is grown.

The disease annually causes a reduction of approximately 2 percent of the total yield of seed flax in the United States.

Varieties of flax may react in five different ways to rust in the field, such varieties being distinguished by the type of uredium produced. Flax may be immune, highly resistant, resistant, incompletely susceptible, or completely susceptible to rust, the various classes being designated by the numerals 0, 1, 2, 3, and 4, respectively.

The development of flax rust within the tissues of a susceptible variety was studied and illustrated in detail.

Histological studies of uredial types characteristic of the five classes of rust reactions indicate that the physiology of the host may influence the resistance or susceptibility of the variety to rust.

Plant extracts obtained from varieties of flax differing in their reaction to rust supported the vegetative growth of urediospores of *Melampsora lini* in accordance with the resistance or susceptibility of the varieties from which they were obtained.

There are no gross anatomical features of the flax plant which may be considered to operate alone in the determination of flax rust resistance.

The thickness of the epidermal membrane of leaves and stems of different flax varieties may be of considerable importance in uredium formation and urediospore liberation. Determinations by means of a modified Joly balance of the pressures required to puncture the stem and leaf epidermis showed that varieties resistant or immune to rust possessed an epidermis which required significantly more pressure to effect puncture than did the epidermis of certain susceptible varieties.

The resistance of the epidermal membrane to puncture is correlated with the possession of a cuticle, the development of a hypodermis, and the isodiametric shape of the individual epidermal cells. The epidermis of susceptible varieties, in general, lacks a well-developed cuticle, the individual epidermal cells are rectangular rather than isodiametric, and the hypodermis is usually absent. The strength of the epidermal membrane as indicated by resistance to puncture may determine the ability of the rust fungus to develop uredia and to liberate its urediospores in certain varieties. Such a condition may reduce the amount of available inoculum produced in the course of the summer.

The size, shape, and arrangement of the cortical fibers cannot be correlated in any way with rust resistance.

Certain flax varieties susceptible to rust possess a larger number of stomata per square millimeter than certain other varieties resistant to or immune from rust. This may be of significance in influencing the percentage of germ-tube penetration.

Stomatal movements may operate in rust resistance. The stomata of the variety Bison, resistant to rust in the field, did not open until after the dew had disappeared from the leaves. This may account for the low percentage of rust development on this variety in the field.

The presence of *Fusarium lini* in the soil may account for the failure of flax rust to appear on the varieties growing in wilt-sick soil, possibly because of the atrophy of the guard cells and the failure of the stomata of these varieties to function.

Darkness during the incubation period apparently suppressed the development of rust. This fact may furnish additional evidence in support of the theory that stomatal movements play a role in the resistance of certain flax varieties to rust.

The application of different nutrient salts influenced rust development. The application of excess nitrogen and excess phosphate increased rust development, while excess potassium appeared to suppress it somewhat. The reaction of a normally susceptible variety was not significantly altered by the application of different nutrient salts, but the development of rust on the normally resistant varieties Buda and Redwing was greatly increased when excess nitrate and excess phosphate were applied.

The resistance of cultivated flax to flax rust cannot be attributed to any one single factor operating alone but must be considered as due to a number of factors operating in conjunction with each other.

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RESPONSE OF WHEAT VARIETIES TO DIFFERENT FERTILITY LEVELS[1]

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INTRODUCTION

The adaptability of a variety of any of our common crops is definitely limited. Climate is probably the most important single factor in setting the boundaries within which a variety may be successfully grown. To weather conditions also are attributable in large measure the great variations in yield between one season and another. Within a given region, type and condition of soil become important, and in some cases are deciding factors in the choice of the strain to be grown.

The question arises: Is it reasonable to expect a winter wheat, developed and tested under conditions of rather high fertility, to maintain its relative standing when grown on soils of medium or low fertility, even of the same general type? Some selections may be better adapted to rich and others to poor soils. Obviously, this has a direct bearing on wheat-breeding and testing programs.

The present study is concerned with the response of a number of wheat varieties and strains to different fertility levels. Since the experiment extends over a 5-year period, a considerable amount of the variation in yield is due to season. For this reason, a rather critical review of the climatological data is necessary. Since, however, it is merely sought to eliminate, as far as possible, the responses to climate and study the responses to fertility levels alone, a literature review on this topic is not considered necessary. A large number of citations may be found in Hannay's bibliography of the influence of weather on crops (*3*).[3]

REVIEW OF LITERATURE

The study of differential reaction of varieties to fertility level has received little attention. Fertilizer tests are generally confined to one or two varieties, and variety tests are usually conducted at only one fertility level. A few experiments, however, do bear directly upon the problem in hand. Weigert and Fürst (*9*) make the point that farmers should choose varieties on the basis of their reaction under the conditions existing on their particular farms. This recommendation is based upon results of tests with winter rye, winter wheat, spring barley, and oats which showed differential response to fertilization. Gregory and Crowther (*2*) ran carefully controlled pot experiments with five pure line varieties of two-rowed barleys varying not

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[2] The writers wish to thank G. H. Stringfield not only for the data of the first years of the fertility-level experiment, but also for most helpful suggestions and criticism in the preparation of this manuscript. They also desire to acknowledge the courtesy of Dr. G. W. Snedecor who read the manuscript and indicated some changes in the statistical treatment.

[3] Reference is made by number (italic) to Literature Cited, p. 142.

only in available nutrients but also including certain "deficiency" series where one essential element—nitrogen, phosphorus, or potassium—was supplied in less than optimum amount. They found a differential response to change of manuring, and conclude that varietal and manurial trials should be combined. They also note that the possibility exists of developing varieties suited to deficient soil types. Beaven, in an appendix to this article, confirms the general conclusions from results of his field trials.

Other less specific references are also to be found. Hunter and Leake (*4*), in discussing British wheats, point out that Yeoman will rank in yield with the best of the softer varieties only when grown on soil in a high state of fertility. They further state: "No hard wheat has been obtained yet that is equal to Squarehead's Master on soils in a medium condition of fertility" (*4, p. 23*). Referring to the Dutch variety Wilhelmina, they say: "* * * it is a prolific grain producer under what may be termed average soil conditions * * *" (*4, p. 31*). In discussing Swedish wheats, they point out that, among spring wheats from progeny of Kolben and Extra-Kolben × Schlanstedter (a variety popular in Saxony), lines were obtained which "* * * were characterized by a distinct tendency towards the requirement of special conditions to enable them to produce their maximum results * * *" (*4, p. 38*). The exact conditions referred to, however, are not stated.

Immer, Hayes, and Powers (*5, p. 418*) made a study of the adaptation of barley varieties, from which they concluded that—

> some varieties reacted differently in different stations, in different years, and in certain stations in specific years but * * * some varieties were significantly superior to others in spite of the differential responses.

Mooers (*6, p. 800*), studied the reaction of a number of corn varieties to two levels of fertility and concluded that—

> Varietal trials on soils of both high and low productivity are required in order to furnish a comprehensive picture of the adaptability and relative standing of different varieties.

Stringfield and Salter (*8*), reporting on the corn crop in the same rotation with which the present paper is concerned, found a differential response of variety to season and to fertility level.

Other citations might be made, but the foregoing indicate that the problem of varietal response to fertility level is one worthy of attention wherever the results of variety tests are to be applied to a wide range of conditions.

OUTLINE OF EXPERIMENT

A 3-year rotation of corn, oats, and winter wheat was started in 1928 at the Ohio Agricultural Experiment Station, Wooster, to study the response of a number of varieties of these crops to different fertility levels.[4] Wheat only is considered in this paper.

The plots were laid out in three blocks, separated by short distances, but all were located on Canfield silt loam. Conditions of slope and drainage varied somewhat. Since the Canfield silt loam was naturally infertile, it was possible to build up a series of fertility levels by adding fertilizers and manure. Level A, receiving no

[4] This experiment was set up by R. M. Salter and G. H. Stringfield. Stringfield selected the strains and varieties of wheat grown and had direct charge of the experiment until 1931 when it was turned over to C. A. Lamb, appointed to the staff at that time.

plant nutrients in any form, was poorest. Levels B, C, and D provided increasingly productive conditions obtained by adding one, two, and four increments of fertilizing materials. The applications are given in table 1.

TABLE 1.—*Plot treatments at the 4 fertility levels used in 3-year rotation tests with corn, oats, and wheat*

Level	Treatment used for crop indicated		
	Corn	Oats	Wheat
A	None	None	None.
B	4 tons manure, 100 pounds 0–16–0 broadcast, 100 pounds 4–12–4 in hill.	do	200 pounds 2–14–4 in fall, 50 pounds nitrate of soda in spring.
C	8 tons manure, 200 pounds 0–16–0 broadcast, 200 pounds 4–12–4 in hill.	do	400 pounds 2–14–4 in fall, 100 pounds nitrate of soda in spring.
D	16 tons manure, 400 pounds 0–16–0 broadcast, 400 pounds 4–12–4 in hill.	do	800 pounds 2–14–4 in fall, 200 pounds nitrate of soda in spring.

The four levels were arranged on adjacent strips and the varieties sown across them. Wheat was planted in triplicate, the size of plot on each level being 5½ by 35 feet (0.00427 acre). Plots were harvested separately; the sheaves were weighed before and the grain after threshing. Weight per bushel was determined for each variety at each level.

PRESENTATION OF DATA

From 15 to 17 winter wheats were grown each year for harvest from 1929 to 1933, inclusive. Of the 20 varieties and strains used, 11 were grown all 5 seasons. Table 2 gives the grain yield, straw yield, and weight per bushel for these 11 lines. Through an error, the sheaves were not weighed before threshing in 1930, and grain yield alone was available for that year. Figure 1 presents graphically the averages for the 11 lines.

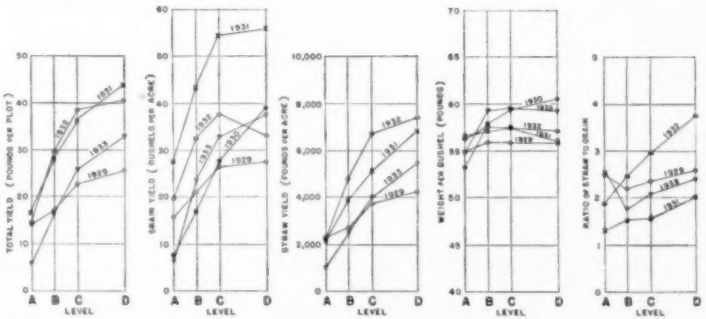

FIGURE 1.—Average response of 11 varieties of winter wheat to 4 soil-fertility levels in 5 seasons.

The plots were cut with an ordinary grain binder, so that uniform stubble was left on the field. This, of course, affected the straw

yields, making them relatively too low on level A, and, in cases where the straw was leaning somewhat, also on level D.

From a study of figure 1, it is clearly evident that season plays an important part in the behavior of winter wheats.

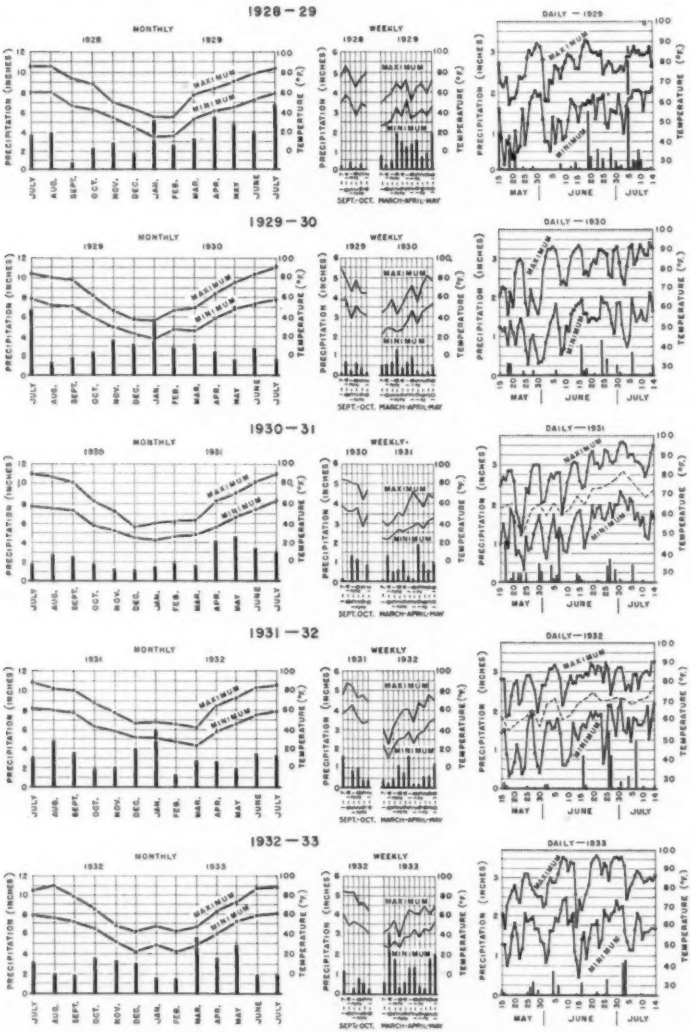

FIGURE 2.—Meteorological data for the period of the experiments, Wooster, Ohio, 1928–33.

Climatological data are presented in figure 2. The graphs show the mean maximum temperatures, mean minimum temperatures, and the precipitation by months for 13-month periods, July to

July, inclusive. In addition, these data are given in greater detail for periods of especial importance to the wheat plant; viz, weekly before and after seeding time and again during the spring growing period, and daily from heading to harvest.

DISCUSSION OF DATA

Variations in yield from one season to another are due largely to climatic factors. Other considerations, certainly of minor importance, include the differences between soil blocks, and on the higher fertility levels in the later years, the residual effects of fertilizers, especially the stable manure applied to the corn.

To obtain maximum yields there must be sufficient moisture and nutrients available to produce strong, healthy fall growth in order that the wheat plants may withstand the winter and be in condition to make a quick start in the spring. During the spring the plants must make the remainder of their vegetative growth and take from the soil the great bulk of the mineral elements which are utilized later in grain development.

At Wooster, factors of prime importance include moisture relations in the fall and early spring, temperature during these periods and also during the winter, and weather conditions at critical periods, such as heading time and the final desiccation period when the kernels are hardening.

Winter injury did not occur to any appreciable extent in any year under consideration. Lodging was not serious, even on level D with heavy applications of nitrate of soda. Hence, these factors need not be considered as influencing the results.

A brief review of weather conditions and crop responses in each of the five seasons as an aid to the interpretation of the statistical analysis of the data follows. The average of the 11 strains grown in all seasons (table 2 and fig. 1) is used as a basis for these observations.

THE 1928–29 CROP

In 1928 September was exceptionally dry, and in October there was only normal rainfall. Wheat did not make good fall growth, and, therefore, could not have taken up any large amount of nutrients before the onset of the dormant period. Spring rainfall in 1929 was very heavy, and probably resulted in some leaching. This was all the more serious since the plants had had an unfavorable start in the fall. Whether this accounted entirely for the lack of response to fertilizers in the 1929 crop is a question, but probably it was the most important consideration. The fact that wheat was the second crop of the rotation to be grown on this particular block and that there was no residual effect of manure and fertilizers used on the corn might have had some influence.

On level A, 1929 yields were low and the average increase from level A to level D was only 12.1 bushels of grain and 1,940 pounds of straw per acre. Straw to grain ratio and weight per bushel showed less variation across the levels than in any other season. This was not surprising in view of the small differences in yield.

TABLE 2.—*Yield per acre and weight per bushel of 11 varieties of winter wheat grown at 4 levels of soil fertility, 1928–29 to 1932–33, inclusive*

Variety [1]	Level	1928–29			1929–30		1930–31			1931–32			1932–33			5-year average		
		Grain	Straw	Weight per bushel	Grain	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw	Weight per bushel	Grain	Straw [2]	Weight per bushel
		Bu.	*Lb.*	*Lb.*	*Bu.*	*Lb.*	*Bu.*	*Lb.*	*Lb.*	*Bu.*	*Lb.*	*Lb.*	*Bu.*	*Lb.*	*Lb.*	*Bu.*	*Lb.*	*Lb.*
Michikof	A	19.2	2,420	59.2	2.7	40.0	24.5	2,640	57.8	14.8	1,800	59.2	3.4	700	54.0	12.9	1,890	54.0
	B	23.3	2,940	59.3	8.3	56.3	40.4	4,180	58.8	29.2	4,320	60.4	17.5	2,080	59.6	23.7	3,380	58.9
	C	26.0	3,700	59.7	16.0	57.4	46.3	4,920	58.9	34.2	5,880	60.6	23.2	3,100	60.4	29.1	4,400	59.4
	D	28.0	4,340	59.7	28.4	59.4	40.6	6,200	57.4	22.5	6,820	59.0	29.2	4,660	60.4	29.7	5,505	59.2
T. N. 1030 (Gladden × Trumbull)	A	18.0	2,580	55.0	10.1	55.0	30.1	2,000	54.0	17.4	1,840	55.3	2.8	600	51.8	15.7	1,755	54.2
	B	23.9	2,820	55.8	26.2	57.9	44.8	3,300	56.4	32.4	4,440	57.4	22.4	2,240	58.1	29.9	3,200	57.1
	C	31.5	3,720	56.2	36.6	60.4	54.4	4,680	56.1	39.9	6,400	58.4	36.3	4,080	58.3	39.7	4,720	57.9
	D	37.7	4,440	56.1	43.1	60.9	57.8	6,960	53.7	38.9	7,780	58.0	40.0	5,420	-------	43.5	6,150	[3] 57.1
T. N. 1032 (Gladden × Trumbull)	A	17.4	2,300	57.5	9.6	57.8	23.5	2,080	57.5	19.7	2,320	58.2	7.3	1,080	56.6	15.5	1,945	57.5
	B	23.9	2,800	57.8	13.3	58.2	43.9	3,500	58.5	27.6	4,300	58.4	26.6	2,800	60.4	27.1	3,350	58.7
	C	30.5	3,880	57.8	26.9	60.2	52.8	4,840	58.9	37.9	6,120	58.2	35.0	4,080	60.2	36.6	4,730	59.1
	D	33.1	4,480	57.9	38.2	61.1	62.7	6,660	57.9	32.7	7,720	58.7	41.5	5,740	-------	41.6	6,150	[3] 58.9
T. N. 1047 (Portage × Gladden)	A	16.8	2,320	55.7	9.7	54.0	31.5	2,320	56.4	20.5	2,500	57.1	5.5	1,000	54.3	16.8	2,035	55.5
	B	23.2	3,040	56.1	19.3	58.1	44.0	4,280	57.1	31.8	5,240	58.4	23.3	2,880	59.9	28.3	3,855	57.9
	C	32.9	4,360	55.6	33.1	60.0	56.7	5,940	57.1	37.3	7,320	58.7	32.2	4,400	60.4	38.4	5,505	58.4
	D	26.8	3,800	55.9	45.5	60.7	55.8	7,080	54.5	34.6	7,640	57.9	36.6	5,840	59.5	39.9	6,090	57.7
Trumbull	A	16.2	2,440	55.6	14.0	58.7	29.7	2,180	58.1	20.2	2,140	54.6	10.6	1,340	55.4	18.1	2,025	56.5
	B	21.1	2,940	55.6	26.8	59.2	40.3	4,200	58.5	34.6	4,620	57.0	27.3	2,720	59.4	30.0	3,620	57.9
	C	23.8	3,640	55.2	34.3	60.4	54.8	5,040	58.6	39.4	6,820	55.0	36.1	4,280	59.2	37.7	4,945	57.7
	D	20.1	3,660	55.4	43.9	60.5	56.6	5,480	56.5	32.0	7,420	56.1	38.0	5,380	58.5	38.1	5,485	57.4
Fulhio	A	16.0	2,280	54.8	11.2	59.0	29.6	2,500	58.2	19.2	2,340	57.6	6.6	980	53.2	16.5	2,025	56.6
	B	19.9	2,580	56.2	19.7	59.3	47.7	4,140	55.8	29.9	5,520	58.3	25.3	2,460	59.6	28.5	3,675	57.8
	C	23.0	3,520	55.9	32.0	60.4	57.1	5,360	59.6	35.1	6,760	58.2	38.5	4,200	59.2	37.1	4,960	58.7
	D	24.4	4,080	54.6	43.8	61.2	59.8	6,980	56.9	32.0	7,420	57.9	43.7	5,700	58.6	40.7	6,045	57.8
T. N. 1049 (Portage × Gladden)	A	14.9	2,420	53.9	5.0	51.7	27.8	2,140	56.9	20.7	1,880	54.1	7.3	1,080	56.3	15.1	1,880	54.6
	B	18.5	2,840	55.0	15.8	57.1	39.7	3,920	57.0	33.2	4,540	55.1	21.3	2,580	59.2	25.7	3,470	56.7
	C	22.0	3,520	54.3	30.0	59.2	53.6	5,420	57.3	40.5	6,360	56.1	30.6	4,140	59.3	35.3	4,860	57.2
	D	21.8	4,100	54.2	35.0	60.5	52.2	7,280	56.3	36.9	6,880	54.3	31.8	5,520	-------	35.5	5,945	[3] 56.3
T. N. 1006 (Portage × Fulcaster)	A	14.8	2,220	52.8	5.4	55.8	29.1	2,060	55.8	27.5	2,560	56.5	8.4	1,040	55.4	17.0	1,970	55.3
	B	19.1	2,680	54.2	16.5	57.1	43.8	4,060	56.3	35.4	6,120	57.9	26.6	2,660	59.3	28.3	3,880	57.0
	C	24.3	3,660	54.6	28.1	59.2	57.2	4,580	56.5	37.2	7,880	57.8	33.5	4,460	59.4	36.1	5,145	57.5
	D	25.2	4,060	53.7	40.1	59.9	64.0	6,500	55.4	27.8	8,280	57.4	45.0	5,720	59.2	40.4	6,140	57.1
T. N. 1034 (Velvet Gipsy × Gladden)	A	13.9	2,080	53.2	3.3	55.3	26.2	1,700	55.2	22.9	2,040	53.2	7.2	1,080	55.4	14.7	1,725	54.5
	B	20.9	2,660	55.1	13.6	56.9	41.3	3,500	55.7	38.5	4,620	55.2	23.1	2,380	58.8	27.5	3,290	56.3
	C	28.5	3,480	54.7	17.4	58.6	53.1	5,220	55.8	41.9	6,900	54.5	32.1	4,100	58.7	34.6	4,925	56.5
	D	30.2	4,580	54.1	36.8	59.9	56.9	7,020	54.4	36.8	7,520	53.8	32.7	5,680	-------	38.7	6,200	[3] 55.6
Junior No. 6	A	10.9	1,920	52.7	2.3	44.0	25.9	1,820	55.7	14.6	2,000	55.9	3.4	540	54.6	11.4	1,570	52.6
	B	16.6	2,400	52.8	7.9	57.1	44.7	3,840	55.8	33.1	4,080	57.5	19.0	1,600	58.4	24.3	2,980	56.3
	C	23.5	3,360	55.0	20.8	57.9	53.9	4,820	56.0	30.6	6,560	57.3	27.5	3,140	59.1	31.3	4,470	57.1
	D	24.2	4,320	55.4	34.1	59.2	56.0	7,260	55.4	36.1	7,020	58.1	38.7	4,660	58.9	37.8	5,815	57.4

T. N. 1045 (Portage × Gladden)	A	10.3	2,100	53.5	9.1	54.2	29.5	2,200	57.4	18.1	2,740	58.1	6.4	1,020	56.6	14.7	2,015	56.0
	B	17.9	2,140	56.2	17.2	59.1	45.4	4,040	58.4	31.4	5,040	58.5	24.3	2,740	60.9	27.2	3,490	58.6
	C	22.8	3,700	56.7	29.5	61.2	57.2	5,780	57.5	38.4	6,720	57.9	33.4	4,160	61.1	36.3	5,060	58.9
	D	29.9	4,660	56.4	41.7	61.8	54.3	7,300	57.3	34.4	7,820	57.9	40.4	5,540	--------	40.1	6,345	[2] 58.4
Average	A	15.3	2,280	54.9	7.5	53.2	27.9	2,140	56.6	19.6	2,200	56.3	6.3	960	54.9	15.3	1,895	55.2
	B	20.8	2,720	55.8	16.8	57.8	43.3	3,900	57.1	32.5	4,800	57.6	23.3	2,460	59.4	27.3	3,470	57.5
	C	26.3	3,680	56.0	27.7	59.5	54.3	5,140	57.5	37.5	6,700	57.5	32.6	4,020	59.6	35.7	4,885	58.0
	D	27.4	4,220	55.8	39.1	60.5	56.1	6,800	56.0	33.2	7,460	57.2	37.7	5,440	[3] 59.2	38.7	5,985	57.6

[1] T. N. indicates the test number under which new lines are carried at Wooster.

[2] 4-year average only.

[3] Average of 6 varieties only.

THE 1929–30 CROP

The fall of 1929 was more favorable than that of 1928, especially in respect to moisture relations, and wheat made much better growth. Spring rainfall in 1930 was light but apparently sufficient for a marked response to fertilizer treatments. The untreated plots on level A gave an even smaller crop than in the previous year, but level D yielded 31.6 bushels more grain per acre than did level A. Straw yields were not available for this crop. Level A gave the lowest and level C the highest weight per bushel found over the entire 5-year period.

THE 1930–31 CROP

The season of 1930–31 was the most favorable encountered and appeared to approach ideal conditions for winter wheat. There was excellent fall growth, a dry winter season, and sufficient spring rainfall to give maximum response to the plant nutrients added. The winter was mild and the spring early. Even level A produced a fair crop; in fact, it yielded more grain than did level D in 1929.

In 1931 level D gave 28.2 bushels of grain and 4,660 pounds of straw per acre more than level A. Grain yield followed the general trend of the total yield quite closely, giving a small uniform increase in the straw to grain ratio from level A to level D. Weight per bushel dropped abruptly from level C to level D.

THE 1931–32 CROP

The yields in 1932 were most erratic. The fall of 1931 was exceptionally mild and wheat made phenomenal growth. The coldest month of the winter was March, when wheat normally resumes its active growth. A severe and sudden frost early in the month killed back the tops considerably and another cold snap later caused slight injury after growth had begun. A third frost occurred at heading time. Either this last frost or the very dry soil conditions then prevailing, or a combination of these factors, resulted in some blasting of the heads. This may partially account for the lower grain yields on level D, since this series heads later than the others. It can hardly be the whole explanation, however, since the dates of heading of levels C and D overlap and level D was consistently low in grain yield in 1932.

The total yield in 1932 followed that of 1931 very closely, except for a decidedly smaller increase for level D over level C. Grain yields, however, behaved very differently. Twelve of the sixteen varieties grown gave less wheat on level D than on level C. Level C yielded 17.9 bushels of grain and 4,500 pounds of straw more per acre than level A. Level D gave 5,280 pounds of straw more than level A. Straw to grain ratio was, of course, high on level C and reached 3.76 on level D. A very high proportion of the dry matter in the total yield of level D was recovered in the straw, as compared with that recovered in other seasons. No study of the heads was made, so that it was not determined whether the lower grain yield was due to fewer heads, fewer kernels per head, or poorer filling of the kernels. The weight per bushel would not indicate shriveled grain. There were no significant differences in length of straw and no lodging on levels C and D.

THE 1932–33 CROP

A more normal crop season occurred in 1932–33. During February there was one cold snap with subzero temperature, but this caused no damage except to tops. Fall conditions were favorable, winter precipitation light, and early spring rainfall probably excessive, followed by a dry period from the middle of May to harvest time. It probably was due to this dry spell and also to the high March precipitation, which may have caused some leaching of nitrates, that yields were reduced as compared to those of 1931.

In 1933, in both total yield and grain yield, the performance paralleled very closely that of 1931, except that all yields were distinctly lower. Level D gave 31.4 bushels of grain and 4,480 pounds of straw per acre more than level A, indicating marked response even to heavy fertilizer applications. With the exception of level A, on which yields were very low, weight per bushel was high and varied little from level to level.

ANALYSIS OF VARIANCE

From a general consideration of the data, it was evident that seasonal factors operating in Ohio influenced yield markedly. The differences between the yearly averages were greater than those between varieties in any one season. Varietal response, however, also appeared to be a factor. To test the validity of this assumption and to obtain some idea of the significance of the variations noted, analyses of variance were made, according to the method developed by Fisher (*1*), first for total yields (that is, the weight of the sheaves before threshing) and again on the grain yields. To estimate the odds for significance, the direct ratio of the variances, Snedecor's F (*7*) was used in place of Fisher's Z.

The results of the analysis of total yield for individual seasons are presented in the first part of table 3. Four years' data only were available. Variety, level, replication, and the variety-level interaction were considered. The analysis was made from the yield in pounds per plot and the figures were rounded off to the nearest pound.

In both total yield and grain yield some plot yields were missing. These were compensated for by using the actual number of plots in each group when dividing the sums of squares. For example, in the total yield for 1930–31, 2 of the 11 varieties were sown in only two replications. In calculating the variance due to variety, nine variety sums were squared, added, and divided by 12, while the other two variety sum squares were added and divided by eight. The two mean values thus obtained were added to give the total sum of squares for variety.

The combination of poor season and lack of response to treatments already discussed had a marked effect on the 1929 results. F values for that year are low because of the small differences between high- and low-level plots, and not because of an abnormally high unaccounted-for error. Even in this season variance due to variety, level, and replication was significant.

In all seasons there were significant differences between varieties, and very significant differences between levels, and between replications, excepting only in 1928–29. The variety-level interaction was significant only in 1930–31, the most favorable season. This suggests

TABLE 3.—*Analysis of variance, by seasons, of total yield and for grain yield for 11 varieties of winter wheat grown at four soil fertility levels in four and five seasons*

TOTAL YIELD

Season	Variance due to—	Degrees of freedom	Sums of squares	Variance	*F*	1-percent point for *F*	5-percent point for *F*	
1928–29	Variety	10	209	20.9	2.71	2.72	2.04	
	Level	3	2,795	931.7	121.00	4.01	2.71	$\bar{x}$=19.7
	Replication	2	51	25.5	3.31	4.85	3.10	σ= 2.77
	Variety-level interaction	30	219	7.3	.95	2.00	1.64	C.V.=14.1%
	Error	86	658	7.7				
	Total	131	3,932					
1930–31	Variety	10	214	21.4	3.89	2.74	2.06	
	Level	3	12,407	4,135.7	751.95	4.04	2.72	$\bar{x}$=30.9
	Replication	2	144	72.0	13.09	4.88	3.11	σ= 2.35
	Variety-level interaction	30	320	10.7	1.95	2.03	1.65	C.V.= 7.6%
	Error	78	431	5.5				
	Total	123	13,516					
1931–32	Variety	10	566	56.6	5.24	2.72	2.04	
	Level	3	14,169	4,723.0	437.31	4.01	2.71	$\bar{x}$=30.6
	Replication	2	298	149.0	13.80	4.85	3.10	σ= 3.29
	Variety-level interaction	30	146	4.9	.45	2.00	1.64	C.V.=10.8%
	Error	86	931	10.8				
	Total	131	16,110					
1932–33	Variety	10	578	57.8	8.38	2.72	2.04	
	Level	3	13,599	4,533.0	656.96	4.01	2.71	$\bar{x}$=20.3
	Replication	2	174	87.0	12.61	4.85	3.10	σ= 2.63
	Variety-level interaction	30	198	6.6	.96	2.00	1.64	C.V.=13.0%
	Error	86	595	6.9				
	Total	131	15,144					

GRAIN YIELD

1928–29	Variety	10	1,086	108.6	6.58	2.58	1.97	
	Level	3	3,088	1,027.0	62.24	4.04	2.72	$\bar{x}$=22.5
	Replication	2	310	155.0	9.39	4.88	3.11	σ= 4.06
	Variety-level interaction	30	549	18.3	1.11	2.02	1.64	C.V.=18.0%
	Error	82	1,349	16.5				
	Total	127	6,382					
1929–30	Variety	10	2,788	278.8	20.96	2.59	1.98	
	Level	3	16,254	5,418.0	407.37	4.07	2.74	$\bar{x}$=22.1
	Replication	2	401	200.5	15.08	4.92	3.13	σ= 3.65
	Variety-level interaction	30	646	21.5	1.62	2.07	1.66	C.V.=16.5%
	Error	72	956	13.3				
	Total	117	21,045					
1930–31	Variety	10	1,049	104.9	4.20	2.58	1.97	
	Level	3	15,918	5,306.0	212.24	4.04	2.72	$\bar{x}$=45.4
	Replication	2	1,100	550.0	22.00	4.88	3.11	σ= 5.00
	Variety-level interaction	36	675	22.5	.90	2.02	1.64	C.V.=11.0%
	Error	78	1,947	25.0				
	Total	123	20,689					
1931–32	Variety	10	687	68.7	2.48	2.58	1.97	
	Level	3	5,422	1,807.3	65.25	4.04	2.72	$\bar{x}$=31.0
	Replication	2	347	173.5	6.26	4.88	3.11	σ= 5.24
	Variety-level interaction	30	752	25.1	.91	2.02	1.64	C.V.=16.9%
	Error	77	2,132	27.7				
	Total	122	9,340					
1932–33	Variety	10	1,240	124.0	7.95	2.55	1.95	
	Level	3	19,003	6,334.3	406.04	4.02	2.71	$\bar{x}$=24.9
	Replication	2	237	118.5	7.60	4.86	3.10	σ= 3.95
	Variety-level interaction	30	511	17.0	1.09	1.96	1.60	C.V.=15.9%
	Error	85	1,330	15.6				
	Total	130	22,321					

that as conditions approach the ideal, varietal response to level becomes a more and more important consideration. Evidence for this is not entirely convincing, however.

In the second part of table 3 is presented a similar analysis for grain yields. Five seasons' results were available. Plot yields to the nearest bushel per acre were used as a basis for this study. Two plots gave less than 0.5 bushel and in these cases a yield of 0 was given and included in the n values.

The 1928–29 results, as was the case with total yields, are less significant than are those of other seasons. Variety-level interaction is in no case significant and shows little uniformity from season to season. If any relationship exists it must be complex, for there is poor agreement between the significance of the interaction for total yields and for grain yields in any season.

The generally lower values for F in the second part of table 3 are due in large measure to the relatively larger unaccounted-for error, as is evident from the larger coefficients of variability. This would indicate factors influencing the filling of the grain which did not affect total yield, or at least not to as great an extent. Threshing errors were probably responsible for part, but certainly not for all this increased error.

In total yield was represented the complete dry matter of the plant, while in the grain was found only that portion translocated during the filling period. The amount and rate of this translocation was dependent, in part at least, upon season and was restricted to a definite and rather short period in the life history of the wheat plant. Since level of fertility influenced dates of heading and ripening, even in one season varieties may have differed in environment for heading, filling, and ripening. Greater variability was, therefore, to be expected in grain yields than in total yields. In other words, there were factors operating which determined whether the dry matter synthesized by the wheat plant was recovered in the straw or in the grain at harvest time.

The data were analyzed, all seasons together, for total yield and also for grain yield. When these analyses were made from the individual plot yields, variance due to replication could not be removed because three different soil blocks had been used. This materially increased the unaccounted for error and resulted in lower statistical significance. By using the mean yields of the three replicates of each variety at each level in each season as a starting point, variance due to replication did not enter into the results, and the difficulty was in part overcome. The fact that the variance due to variety, level, season, and the three interactions as presented in table 4 is roughly one-third that obtained when the calculations were made from individual plot yields indicates that no serious error was introduced by using the mean values. The analyses presented in table 4 are thus justified.

From table 4 it is seen that insofar as total yield is concerned, varieties respond differently to level of fertility and to season. This table shows that these interactions are also significant when grain yields alone are considered.

TABLE 4.—*Analysis of variance for total yield and grain yield of 11 varieties of winter wheat grown at 4 levels of soil fertility during 4 and 5 seasons*

TOTAL YIELD (4 SEASONS)

Variance due to—	Degrees of freedom	Sums of squares	Mean square (variance)	*F*	1-percent point for *F*
Variety	10	241.01	24.10	15.55	2.55
Level	3	13,510.75	4,503.58	2,905.53	4.01
Season	3	5,130.97	1,710.32	1,103.43	4.01
Variety-level interaction	30	139.94	4.66	3.01	2.00
Variety-season interaction	30	293.72	9.79	6.32	2.00
Level-season interaction	9	1,259.68	139.96	90.30	2.66
Total accounted for	85	20,576.07			
Error	90	139.88	1.55		
Total for experiment	175	20,715.95	$\bar{x}$=25.416 pounds per plot σ=1.24 pounds C. V.=4.88 percent		

GRAIN YIELD (5 SEASONS)

Variety	10	1,204.68	120.47	18.59	2.49
Level	3	18,081.27	6,027.09	930.10	3.94
Season	4	16,336.56	4,084.14	630.26	3.47
Variety-level interaction	30	348.23	11.61	1.79	[1] 1.94
Variety-season interaction	40	1,316.14	32.90	5.08	1.94
Level-season interaction	12	2,924.82	243.74	37.61	2.33
Total accounted for	99	40,211.70			
Error	120	777.68	6.48		
Total for experiment	219	40,989.38		$\bar{x}$=29.3 bushels per acre σ=2.55 bushels C. V.=8.70 percent	

[1] 5-percent point for F=1.60.

DIRECT COMPARISON OF VARIETIES

The primary reason for establishing the fertility-levels experiment was to determine whether or not varieties differed from one another in their response on poor as compared with rich soils and particularly to obtain information on the reaction of new lines that the station was developing. A large proportion of the varieties and strains included were new selections. The wheats grown were all reasonably well adapted to conditions in Ohio, and, therefore, the significance of the differences cannot be attributed to the inclusion of some varieties which could not react normally at Wooster. The analyses of variance indicated, however, that real differences in the behavior of these varieties did exist. It will be interesting, therefore, to compare the performance of one of the highest yielding Ohio lines, T. N. 1006 (Portage × Fulcaster), and that of Michikof, an Indiana variety. The data are presented graphically in figure 3. The actual figures are found in table 2.

In total yield the same general trend is noted in both varieties. For the 1929 crop the two curves are very similar. In the other three seasons for which data are available, T. N. 1006 appeared to be able to make better growth than Michikof at the higher levels of fertility, and the differences became greater as the available nutrients increased.

This is the type of differential response of variety to level which apparently accounts for the significant interaction of these factors.

For the grain yields, very similar relationships held. In the crops harvested 1930, 1931, and 1933, T. N. 1006 gave greater response to fertilizer in grain and in total yield as well in the last two of these seasons. Total yields were not available for 1930. In four crops out

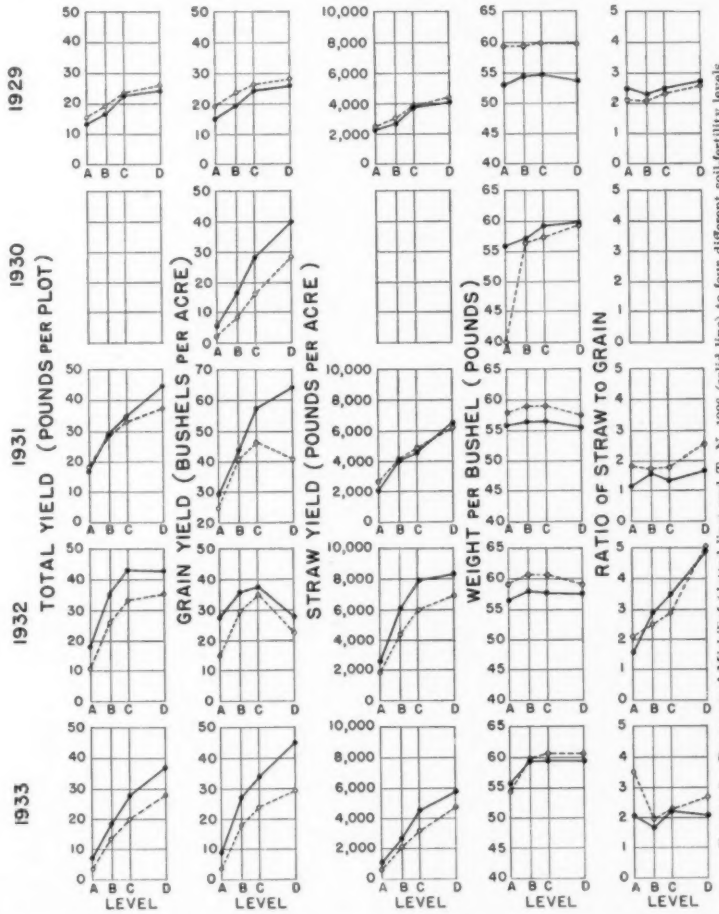

FIGURE 3.—Response of Michikof (dotted line) and T. N. 1006 (solid line) to four different soil-fertility levels during five seasons.

of the five, T. N. 1006 gave a higher grain yield than Michikof at every level. However, in 1929 Michikof consistently produced more grain. It may be significant that this last-mentioned season was the one in which fertilizers had the least effect.

In 1931 T.N. 1006 demonstrated its ability to give very high yields when conditions are suitable. Michikof in the same season dropped in grain yield from level C to level D in all three replications. This

probably indicates a real difference in ability to utilize large amounts of plant nutrients for grain production.

Michikof is a rather hard wheat; T. N. 1006 is soft. In general, hard wheats have not yielded so well as soft at Wooster. Whether there is a connection between class of wheat and response to fertilizer could not be determined from the data at hand.

Michikof showed less variation in weight per bushel than did T. N. 1006, and it was generally heavier. The extremely low yield of Michikof at level A in 1930 and 1933 was undoubtedly the reason for the abnormal bushel weights on these plots. Both wheats followed the average trend and reached a maximum at about level C. Weight per bushel was apparently subject to greater variation in T. N. 1006 than in Michikof, especially between seasons.

SUMMARY AND CONCLUSIONS

A 3-year rotation of corn, oats, and winter wheat has been grown at four fertility levels for five seasons at the Ohio Agricultural Experiment Station. Wheat alone is considered in this paper.

Data on grain yield, straw yield, and weight per bushel are given for 11 wheat varieties which were grown in all five seasons.

Climatological data for the period covering the five wheat crops are presented.

Analysis of variance of total yield and of grain yield indicates that differences due to variety, to fertility level, to season, and to each of the three interactions of these factors cannot be ascribed to errors of random sampling alone. In all but one case the odds for significance exceed 99:1, when all seasons are considered together.

In the analysis of variance, residual error for the grain yields is greater than that for total yields, as shown by the coefficients of variability; this indicates that factors other than those considered influence translocation appreciably. Date of heading and of maturity probably fall in this class, since they result in variations in the environment during the period in which the grain is filling and hardening.

The results obtained indicate definitely that varieties respond differently to a series of fertility levels. Of the varieties used, none was definitely superior at only the high or at only the low levels of fertility. The data, however, do not preclude the existence of such varieties. Further work is being conducted in an attempt to analyze the response to fertility levels into its components.

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ROOT GROWTH OF SEEDLINGS OF PINUS ECHINATA AND PINUS TAEDA[1]

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INTRODUCTION

The operations of growing and transplanting southern pine seedlings for reforestation and afforestation purposes are still beset with the danger of high mortality of transplanted stock. The advantages of fall over spring transplanting, or vice versa, are yet to be determined, and, of course, will be determined only after many years of actual planting in both seasons. In the Southern States where winter growth, or merely semidormancy, of at least some plant parts is apt to obtain, it seems possible that the performance of roots, as regards duration or seasonal periodicity of growth, may be an important factor influencing mortality. The object of this experiment was to investigate the growth of roots of shortleaf and loblolly pine seedlings, with particular reference to the extent and time of elongation.

REVIEW OF LITERATURE

A comprehensive review of the literature pertaining to root growth has been given by Stevens (*7*).[2] However, a few references not included in his publication may be cited.

Petersen's work (*5*) supports the conclusions of others that there may be some degree of winter growth of roots of deciduous trees. Schimper's observations (*6*) led him to conclude that there is periodicity in plant growth, which he attributed to causes within the plant. Engler (*1*) believed that there occur two periods of tree-root elongation, one in the spring, the other in the fall. Brown (*2*) inferred that root growth of *Pinus strobus* ceased in autumn and was resumed in April. MacDougal (*4*), referring to tree growth in general, stated that although continuous cambial activity may be expected in evergreen trees, growth ceased in Monterey pine when the cambial temperature fell below 8° C. Kinman (*3*) observed no winter growth of roots in the case of mature myrobalan, peach, and apricot trees. He found two periods of more active root growth: In February and March, for both myrobalan and peach trees, from mid-September to mid-November for myrobalan, and from mid-October to mid-November for peach, with essential cessation between these periods. The apricot had one wholly inactive period, from late January to late March.

METHOD OF STUDY

Two frames of truncated-wedge shape (fig. 1) were constructed and placed in excavations at the Arkansas Agricultural Experiment Station at Fayetteville. The top of each frame was at the level of the surface of the ground. The sides of the frames were inclined

[1] Received for publication Nov. 29, 1935; issued August 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 149.

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inward from the base at an angle of approximately 18° from the vertical. Each side of a frame accommodated ten 10-inch by 24-inch pieces of double-strength glass[3] which were supported by the base, top, and vertical braces. Hinged, lightproof and rainproof lids were constructed to divert rain water into ditches which led away from the frames, and tile was placed under each box to take off seepage water during rainy periods. Pulverized A_1 horizon Clarksville silt loam was placed around the frames and allowed to settle three times with subsequent refilling. Care was taken at all times to keep the soil layer above the level of the glass to exclude light.

On April 1, 1933, twenty 1-year-old seedlings each of *Pinus echinata* Mill. (shortleaf pine) and *P. taeda* L. (loblolly pine) were planted along the sides of the frames with roots against the glass. On April 26, 1933, the exact position and length of all roots visible was etched

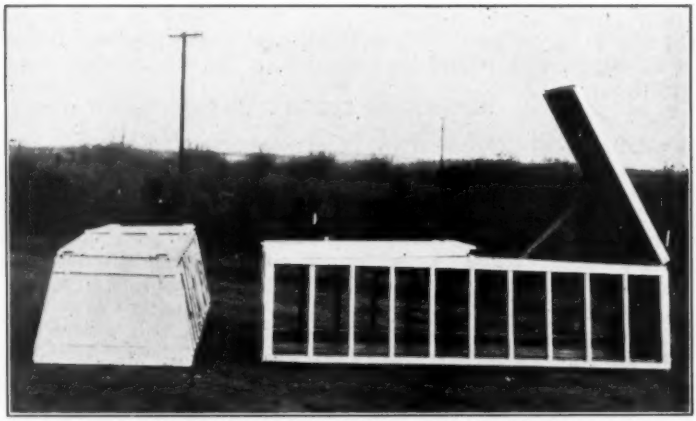


FIGURE 1.—Root-growth observation frames before installation.

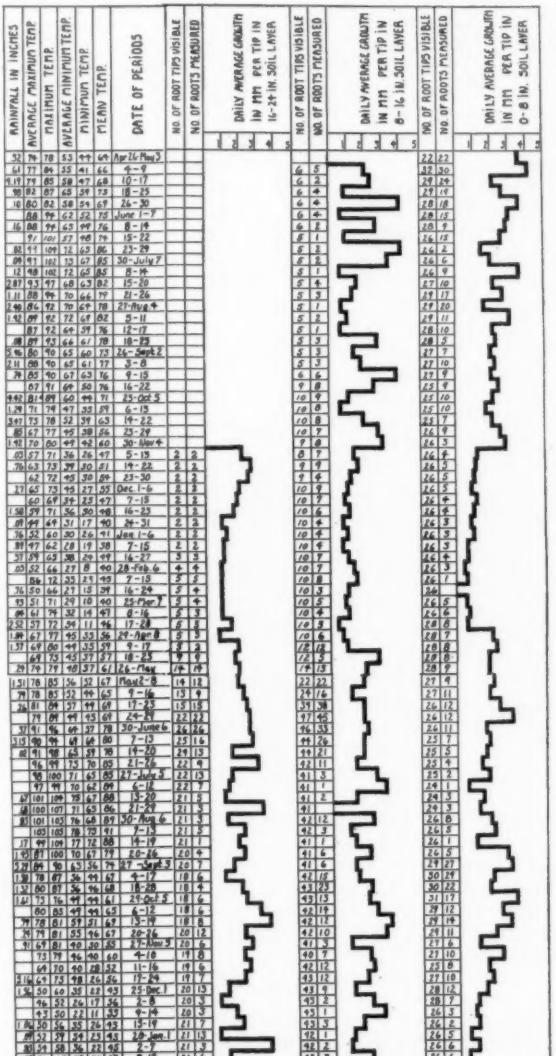
on the interior of the glass with a diamond-pointed pencil. Subsequently, at intervals averaging 8 days, etchings were made of all active roots, with numbers referring to recorded dates, throughout a period of 2 years. At the end of that time the glasses were removed, marked in three vertical intervals of 8 inches each, and the extent of root growth in each of these intervals was determined for the periods of observation. All readings were reduced to daily average growth in length in millimeters for each level for each period. In the records no attempt was made to separate horizontal from vertical growth of roots.

The mean temperature in Fahrenheit and the precipitation for each observation period were determined from the records of the meteorological station situated 600 feet from the frames.

RESULTS

The observed occurrence of continuous growth (fig. 2) during the entire period of 2 years, in at least two levels, is particularly sig-

[3] Six glasses were broken by the pressure of the growing roots. Stronger glass or narrower pieces would doubtless have proved more satisfactory.


RAINFALL IN INCHES
AVERAGE MAXIMUM TEMP.
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MINIMUM TEMP.
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DATE OF PERIODS
NO. OF ROOT TIPS VISIBLE
NO. OF ROOTS MEASURED
DAILY AVERAGE GROWTH IN MM PER TIP IN 16-24 IN. SOIL LAYER
NO. OF ROOT TIPS VISIBLE
NO. OF ROOTS MEASURED
DAILY AVERAGE GROWTH IN MM PER TIP IN 8-16 IN. SOIL LAYER
NO. OF ROOT TIPS VISIBLE
NO. OF ROOTS MEASURED
DAILY AVERAGE GROWTH IN MM PER TIP IN 0-8 IN. SOIL LAYER

FIGURE 2.—Root growth of *Pinus echinata* seedlings from April 26, 1933, to April 17, 1935.

nificant.[4] The absence of records of growth for the upper level for the period of February 16–24, 1934, and for the middle layer for the period of July 21–29, 1934, is probably fortuitous. The fact that the roots of the young seedlings had not yet reached the lowest level accounts for the absence of records in that level between April 26, and November 4, 1933.

Periods occurred in which there were both greater average daily increment to each root and a larger number of growing roots. These were: Late April to early June of 1933, mid-July to mid-August of 1933, early April to early June of 1934, early September to late October of 1934, and early April of 1935. All of these periods of greater growth were coincident with a mean temperature of 55° F. or higher, and with the occurrence of considerable rainfall.

Likewise, periods occurred in which there was relatively little growth. During some of these there was both little daily average increment and a small number of active roots. These periods were: Early November to mid-March of 1933–34, mid-June to mid-August of 1934, and early December to late March of 1934–35. The first- and third-named periods were coincident with mean temperature of 56° F. or lower, and the second with low rainfall. One period, from early June to mid-July of 1933, with low rainfall, showed a conspicuous diminution in the number of active roots, but a less conspicuous lessening of daily average increment. There is evidence of correlation of least number of active roots and lowest daily average increment with periods of lowest average minimum temperature occurring during the 2 years, that is, from November 5, 1933, to March 28, 1934, and from November 11, 1934, to March 1, 1935.

With the resumption of greater activity in the spring there was a noticeable lag both in average daily increment and in the number of active roots in the lower levels as compared to the top.

There is some evidence that prolonged dry periods have a greater retarding effect on root growth in the upper level than in the lower levels. This is observable in the periods of June 1933, and mid-June to mid-July of 1934. In general, the effects of both low temperature and low rainfall were more evident in the topsoil layer than in the lower ones. Conspicuous fluctuations in daily average increment occurred in the middle layer in 1933, but after that the roots in both of the lower levels showed less tendency to vary, at least as regards rate of growth, than those of the top layer. During the winter, or the time when the mean temperature of periods was 53° F. or lower, high rainfall had no accelerating effect on rate of root growth in any of the levels.

DISCUSSION OF RESULTS

The period of greater root activity in the spring is naturally associated with the various phenomena and conditions attending the end of the semidormant winter period and the beginning of the period of active stem growth. A second period of semidormancy occurred during the very warm and dry summer months, followed by a renewal of active growth during the cooler, rainy period of early autumn. It should be mentioned that in both years there was a noticeable recurrence of stem growth during this latter period.

[4] Graphic and tabular representation of growth is for *Pinus echinata* only. There was not enough difference in performance of roots of *P. taeda* to justify separate presentation.

The greater number of records of active roots during or following rainy periods is due both to the fact that new roots are initiated and that inactive roots become active again at such times. The retardation of activity in the top layer during extremely hot, dry weather may be due to reduced soil moisture, and possibly to high, unfavorable temperature of the soil.

Within the limits of the research method, the evidence secured supports the opinion of some earlier investigators that root growth of trees is slow in summer as a result of low moisture content of the soil; in winter due to low temperature; and greater during periods of high soil moisture and favorable temperature. It would probably be an error, however, to wholly dissociate the slow growth, or semidormancy of roots in midsummer from other factors, such as high transpiration, and probably excessively high temperatures of the topsoil layer.

SUMMARY

Roots of seedlings of *Pinus echinata* Mill. and *P. taeda* L. made recordable growth during every 8-day period for 2 years.

Periods of more active growth were observable—in early spring, and late summer-early autumn, and in 1 year, in midautumn.

There were two marked periods of semidormancy; both the number of growing roots and the daily average increment were lower in the period from December 1 to March, and from the end of June through August.

The periods of slow growth, winter and midsummer, were associated with low air temperature and low rainfall, respectively. Greatest activity of roots was associated with considerable rainfall and favorable but not too high air temperature.

There was less growth in the top 8-inch layer than in lower levels during periods of lowest air temperature, and of high air temperature and low rainfall. In general, there was somewhat less variation in the rate of growth of roots in the two lower levels than in the upper one.

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INHERITANCE OF COMPLEMENTARY DWARFING FACTORS IN WHEAT [1]

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INTRODUCTION

The occurrence of dwarfs in cereal and other plant hybrid populations is being reported by an increasing number of investigators. In most cases in the cereals it has been observed in connection with hybridization experiments for economic improvement. The crosses reported in this paper were produced incidentally in a wheat-breeding program, the main object of which was the production of improved bunt-resistant varieties. Complete dwarfing occurred in the F_1 generation of eight crosses, although dwarfs more commonly are found as segregates in the F_2 generation.

The dwarf condition in plants apparently is due to genetic factors, to chromosome abnormalities, or to other causes that interfere with normal height development. It is now generally recognized that dwarfing of the kind usually encountered in intraspecific hybrids is governed chiefly by factor interactions but that the ratios obtained usually are modified to some extent by irregular chromosome behavior.

The presence of dwarfs in wheat crosses which first appear in the F_2 generation has been explained by a number of investigators by the interaction of two factors, a factor for normal height, *N* (or *I*), which when present inhibits the dwarfing factor, *D*. In these experiments the F_2 segregation occurred in a ratio of 13 normal to 3 dwarf plants. Workers whose results with dwarfing have been explained on a two-factor basis include Hayes and Aamodt (*6*),[2] Clark and Hooker (*2*), Goulden (*5*), Stephens (*9*), Stewart and Tingey (*10*), Clark and Quisenberry (*3*), Nieves (*8*), Churchward (*1*), Tingey (*12*), and Waterhouse (*14*).

Other workers, viz, Waldron (*13*), Neethling (*7*), Florell (*4*), and Churchward (*1*), obtained segregations that indicated either three- or four-factor differences but which possibly are best explained as poor fits to the two-factor difference obtained by other workers.

Dwarfs in the F_1 generation from normal parents have been observed by a number of wheat breeders, including Waterhouse (*14*), who found dwarfs in the F_1 generation in 25 wheat crosses. Results from such dwarfs carried through the F_2 and F_3 generations were presented by Thompson (*11*).

These results were explained on the basis of the interaction of three factors. Thompson assumed the presence of the usual factor for normal, or inhibitor for dwarf, *I* (or *N*), with the dwarfing factor (*D*) and an extra inhibitor (*E*), which inhibited or neutralized the action

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[2] Reference is made by number (italic) to Literature Cited, p. 160.

of the inhibitor *I*. Thus, the dwarf plants were conditioned by the genes *IED* and *ieD*, and the normal plants by *IeD* and *IEd*.

According to Thompson's scheme the F_2 population will segregate in a ratio of 39 *D* : 25 *N*. The F_2 normal plants selected at random will produce F_3 families in a ratio of 19 true-breeding normal to 6 segregating normal and dwarf, and the F_2 dwarf plants will produce 7 true-breeding dwarf families to 32 segregating dwarf and normal.

MATERIAL AND METHODS

More than 50 crosses were made at the Idaho Agricultural Experiment Station, Moscow, Idaho, in 1931, by the senior writer and his assistants in a wheat-breeding program designed to develop improved bunt-resistant varieties of white winter wheat for the Pacific coast region. Four of these crosses and their reciprocals produced all dwarf plants in the F_1 generation. Turkey-Florence (G–326W8) was a parent in three of these crosses and Hussar-Hohenheimer (C. I. 10068 [3]) in the other. Dwarfs in the F_1 generation also were obtained in 1933 from two Arco selections (8118 and 8120) crossed with both Turkey-Florence and Hussar-Hohenheimer in 1932. Some F_1 plants of the Hussar-Hohenheimer × Jenkin cross were backcrossed to both parents in 1932.

Turkey-Florence is a white-kerneled, bunt-resistant winter wheat selected from the same cross from which Ridit was produced. The original Turkey-Florence cross was made by E. F. Gaines at the Washington Agricultural Experiment Station, Pullman, Wash., and the white-kerneled Turkey-Florence selection used in the present investigation was made by D. E. Stephens at Moro, Oreg.

Hussar-Hohenheimer (C. I. 10068) was selected by the late H. M. Woolman at Corvallis, Oreg. This wheat is a bearded, red-kerneled, white-strawed strain having a winter habit of growth.

Jenkin is a late-maturing spring variety of club wheat having tall white straw, soft white kernels, and brown chaff. Federation is a medium early spring variety having short stiff straw, soft white kernels, and brown chaff. Baart is an early spring variety having medium-tall, slender, and pliable straw and large white semihard kernels.

The Arco selections were obtained from the cross Arcadian × Hard Federation, which was made by Walter Carpenter at Moro, Oreg., in 1919. The Arco selections have short stiff straw, soft white kernels, and a winter habit with rather low winter hardiness.

Dwarf plants usually can be distinguished readily from normal plants by the much reduced height and by the abundance of grassy leaves at the base of the plants resulting from their shortened internodes. Height measurements of the F_1 and F_2 dwarfs and the parents were taken for comparison, but in the F_3 generation the dwarfs were distinguished by inspection only.

In making the crosses, pollen from a single spike was used to fertilize each emasculated spike. A good set of seed was obtained both in 1931 and 1932. The crossed kernels were sown 4 inches apart in 5-foot rows spaced 1 foot apart, between a row of each of the parents. Fairly vigorous F_1 hybrid plants were obtained in both 1932 and 1933,

[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

although there was some winter-killing of tender strains in both years. In 1932 Baart winter-killed, and a somewhat reduced stand was obtained in both the Federation and Jenkin parents. Good stands were obtained in the F_1 hybrids and vigorous plants were produced in the hybrids as well as in the parents that survived.

The height of each of the hybrid and parent plants studied was obtained by measuring the height of the earliest culm. The length of spike of the earliest culm also was measured and the average weight of kernel in milligrams was determined from the weight of 100 kernels from each plant.

Most of the F_1 plants produced seed fairly abundantly. Dwarf plants that did not produce spikes were designated as grass plants or grassy dwarfs. Seeds from F_1 dwarf and parent plants were sown in late October at the rate of 100 kernels per row in 16-foot rows 1 foot apart. Emergence did not take place before the winter snows began, and fall-stand counts could not be made in 1932.

A good snow covering was present during most of the winter months, but only fair winter survival was obtained. The dwarfs showed most evidence of winter injury. The growing season again was favorable so that vigorous plants were obtained from most of the surviving individuals.

At harvest time, plant height, length of spike, and average weight of kernel per plant were obtained as in the F_1 generation. Seed of good quality again was obtained from most of the dwarfs as well as from the parent plants.

In the fall of 1933, this experiment was transferred to the Pendleton Field Station, Pendleton, Oreg., where the crop was grown and the data were taken by the junior writer. Seed from about 25 to 35 dwarf F_2 plants and from about 35 to 70 normal F_2 plants was selected at random from each of four crosses to continue the experiments in the F_3 generation. About 75 to 100 kernels, in some instances fewer, from each plant were sown in 16-foot rows 1 foot apart. Reasonably satisfactory emergence occurred, but some of the weaker plants were lost during the winter. Limited moisture and hot weather in the spring caused many dwarfs to die prematurely and rendered identification difficult. These conditions also limited the height of some weaker normal plants.

EXPERIMENTAL DATA

THE F_1 GENERATION

The average height of culm, length of spike, and weight of kernel of four dwarf F_1 wheat crosses and of their normal parents, at Moscow, Idaho, in 1932, are presented in table 1. Similar data, except for kernel weights, for four additional crosses, in 1933, are also given.

The average height of the dwarfs in 1932 was about 15 to 17 inches; that of Turkey-Florence 44 inches, of Jenkin 43 inches, and of Federation 33 inches. The standard deviations of the heights of the F_1 hybrids were similar to those of the normal parents but were relatively larger owing to dwarfness of the plants. The mean height of the F_1 dwarfs in 1933 ranged from about 10 to 13 inches. The parents likewise were shorter than in 1932, owing to dry weather at maturity and to poor soil. The F_1 dwarfs and parents of two of these crosses are shown in figure 1.

TABLE 1.—*Average culm height, spike length, and kernel weight of eight dwarf F_1 wheat crosses and their normal parents, at Moscow, Idaho, 1932 and 1933*

Year and parent or hybrid	Plants	Mean and standard deviation		
		Culm height	Spike length	Kernel weight
1932	*Number*	*Inches*	*Cm*	*Mg*
Turkey-Florence	6	45.17± 1.47	11.67±1.92	47.50±1.92
F_1 hybrid	15	16.92±10.40	7.03±2.43	39.77±6.11
Jenkin	9	43.44± 2.87	6.60± .78	45.80±4.40
Turkey-Florence	8	44.63± 2.99	10.94±1.63	46.25±1.66
F_1 hybrid	7	15.43± 2.91	8.50±1.29	37.56±4.32
Federation	11	32.86± 5.45	8.64±1.91	42.73±5.04
Turkey-Florence	6	41.33± 2.83	9.92±1.17	37.00±1.15
F_1 hybrid	7	15.71± 3.57	8.00±1.36	
Baart [1]	0			
Hussar-Hohenheimer	9	39.56± 8.17	11.05±1.69	45.00±3.49
F_1 hybrid	8	14.88± 7.77	9.81±3.39	
Jenkin	2	38.50±10.59	6.00±1.42	47.00±1.42
1933				
Hussar-Hohenheimer	8	33.38± 2.98	8.94± .47	
F_1 hybrid	10	9.70± 2.12	9.15±1.59	
Arco (sel. 8120)	9	28.56± 3.25	7.67±1.86	
Turkey-Florence	1	36.00	10.00	
F_1 hybrid	1	13.00	9.00	
Arco (sel. 8120)	3	30.00± .00	7.88± .41	
Arco (sel. 8118)	3	32.33± 3.11	8.33± .58	
F_1 hybrid	3	12.00± 4.20	7.67± .82	
Turkey-Florence	4	39.00± 2.35	10.25±1.50	
Arco (sel. 8118)	13	29.13± 3.43	8.00±1.21	
F_1 hybrid	13	10.69± 5.30	8.69± .80	
Hussar-Hohenheimer	12	36.58± 2.22	9.33±2.48	

[1] Winter-killed.

The length of the main spike of the F_1 dwarfs was intermediate in some crosses but less than that of either parent in others.

The average weight of kernel is given for only two crosses, but in both cases it is definitely less than in either parent.

The first generation of the Hussar-Hohenheimer × Jenkin backcrosses was grown at Moscow, Idaho, in 1933. The proportion of normal to dwarf plants expected in the backcross to either parent was 1:1. Thirty-five mature plants were obtained from the backcross to Hussar-Hohenheimer, of which 17 were dwarf and 18 normal. This was close to the expected result. Seven plants, all normal, were obtained from the backcross to Jenkin, the number of plants apparently being too small to give the expected segregation.

The mean height of the dwarf plants, not including two grassy plants, was 10.47±5.84 inches, and that of the normal plants 32.94± 5.88 inches. The mean height of the Hussar-Hohenheimer parent (28 plants) was 31.14±8.73 inches, and that of the Jenkin parent (4 plants) 32.00±4.69 inches. The mean height of the 7 normal plants obtained by backcrossing the F_1 with Jenkin was 31.86±9.21 inches.

THE F_2 GENERATION

The number of seeds of the different crosses sown and the plants harvested in the F_2 generation were as follows: Turkey-Florence × Jenkin, 688 sown and 394 (57.3 percent) harvested; Turkey-Florence × Federation, 381 sown and 139 (36.5 percent) harvested; Turkey-Florence × Baart, 366 sown and 147 (40.2 percent) harvested; Hussar-Hohenheimer × Jenkin, 410 sown and 137 (33.4 percent) harvested.

It is evident that all of the crosses suffered serious winter injury, since in favorable seasons survivals of over 90 percent are not uncommon. The data on normal and dwarf plants in the F_2 generation are presented in table 2. According to Thompson's three-factor hypothesis, these crosses should have given dwarf and normal plants in a ratio of 39 *D*:25 *N*. This ratio was indicated only in Turkey-

FIGURE 1.—Dwarf F_1 hybrid and parental plants at Moscow, Idaho, 1933. In foreground: *a*, Hussar-Hohenheimer; *b*, dwarf hybrid; *c*, Arco; *d*, Turkey-Florence; *e*, dwarf hybrid; *f*, Arco.

Florence × Baart, in which the deviation was 3.3 times the probable error. Only in this cross did the number of dwarfs exceed the number of normal plants.

TABLE 2.—*Number of normal and dwarf plants in the F_2 generation in four wheat crosses at Moscow, Idaho, 1933*

Cross	F_3 families	F_2 plants					
		Total	Observed		Calculated[1]		Deviation
			Normal	Dwarf	Normal	Dwarf	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Turkey-Florence × Jenkin and reciprocal	6	394	245	149	154	240	91±6.5
Turkey-Florence × Federation	4	139	83	56	54	85	29±3.2
Turkey-Florence × Baart	3	147	70	77	57	90	13±4.0
Hussar-Hohenheimer × Jenkin and reciprocal	6	211	137	74	82	129	55±4.8

[1] Based upon the ratio of 39 dwarf to 25 normal.

It seems probable that winter-killing destroyed the greater portion of the dwarf segregates. The number of normal plants did not exceed the number that might be expected from a reasonably good survival, whereas the number of dwarf plants was decidedly less. Dwarf plants are less vigorous than normal plants, and therefore more likely to be injured by unfavorable conditions.

The mean height of the dwarfs in the F_2 generation ranged from 12 to 15 inches. A number of somewhat taller dwarfs, which probably represented heterozygotes, were classified as intermediate dwarfs. These had the typical dwarf characteristics in most respects but were 28 to 34 inches in height. There was little or no overlapping in the height of the dwarf, intermediate-dwarf, and normal classes.

THE F_3 GENERATION

Seed from normal and from dwarf F_2 plants was selected at random for growing at Pendleton, Oreg., in the F_3 generation. A fairly large population was produced by the normal F_2 plants, but the dwarf progenies suffered from winter-killing.

The normal F_2 plants, according to the three-factor hypothesis, should produce in the F_3 generation a ratio of 19 true-breeding normal (tall) families to 6 segregating families. Two of the families should segregate in a ratio of 3 normal to 1 dwarf, and 4 families in a ratio of 13 normal to 3 dwarf plants. The dwarf F_2 plants should produce segregations in the F_3 generation of 7 true-breeding dwarf to 32 segregating families. Four types of segregation for dwarf and normal plants should occur among the 32 segregating families in the following ratios: 16, 3 : 1; 4, 13 : 3; 4, 9 : 7; and 8, 39 : 25.

The ratio of true-breeding to segregating families from both normal and dwarf F_2 plants was close to the calculated results (table 3). A study of the relative number of normal and dwarf plants in individual rows (families) from normal F_2 plants showed, however, a deficiency of dwarf plants in nearly all rows, based on the expected ratios 3 : 1 and 13 : 3. A number of these rows from each of the 4 crosses contained only 1 to 4 dwarfs among 50 or more plants. These may be normal progenies in which some chromosomal aberration has resulted in the production of dwarfs. Thompson (*11*) grew F_4 progenies from normal plants selected from four F_3 families, each of which likewise had given but few dwarfs. He found dwarfs in the progeny of but a single plant and concluded that such F_3 rows were in reality true-breeding normals.

TABLE 3.—*Behavior in the F_3 generation of the progenies of F_2 plants of four wheat crosses at Pendleton, Oreg., 1934*

Cross	Progenies of normal F_2 plants						Progenies of dwarf F_2 plants					
		Observed		Calculated [1]				Observed		Calculated [3]		
	Total	Normal	Segregating	Normal	Segregating	Deviation [2]	Total	Dwarf	Segregating	Dwarf	Segregating	Deviation [2]
	No.	*No.*	*No.*	*No.*	*No.*		*No.*	*No.*	*No.*	*No.*	*No.*	
Jenkin × Turkey-Florence and reciprocal	50	40	10	38.0	12.0	2.0±2.04	36	3	33	6	30	3±1.55
Turkey Florence × Federation	61	44	17	46.4	14.6	2.4±2.25	29	1	28	5	24	4±1.39
Turkey-Florence × Baart	51	39	12	38.8	12.2	.2±2.06	33	2	31	6	27	4±1.49
Hussar-Hohenheimer × Jenkin and reciprocal	38	24	14	28.9	9.1	4.9±1.78	25	8	17	4	21	4±1.29
All crosses	200	147	53	152.0	48.0	5.0±4.07	123	14	109	22	101	8±2.87

[1] Based upon the ratio of 19 true-breeding normal to 6 segregating.
[2] Probable errors calculated by the formula $0.6745\sqrt{pqn}$.
[3] Based upon the ratio of 7 true-breeding dwarf to 32 segregating.

If the families having a low number of dwarfs are included with the true-breeding normal families, a very good fit for the 19 true-breeding to 6 segregating lines is obtained.

The number of true-breeding dwarf families was less than the expected 7:32 ratio in three of the crosses and above the expected number in one cross. Deviations from the calculated number were less than three times the probable error in all crosses. The somewhat small number of dwarf families possibly may be accounted for by a higher mortality in the F_2 among the homozygous than among the heterozygous dwarf plants.

When the distributions for the individual rows in the various crosses were examined for conformity to the expected ratios, it was evident that the families could not be separated accurately into the expected types of segregation. However, a number of the families from normal F_2 plants, particularly in Jenkin × Turkey-Florence and Turkey-Florence × Federation crosses, segregated in satisfactory 3:1 and 13:3 ratios. It is clear that the dwarf plants suffered considerable killing.

The F_3 segregations in the Turkey-Florence × Baart cross are shown by individual families in table 4.

TABLE 4.—*Number of normal and dwarf plants in F_3 families from F_2 normal plants in the Turkey-Florence × Baart cross at Pendleton, Oreg., 1934*

Plants from normal F_2 plants				Plants from dwarf F_2 plants			
Normal	Dwarf	Total	Breeding behavior	Dwarf	Normal	Total	Probable breeding group
Number	*Number*	*Number*		*Number*	*Number*	*Number*	
46	17	63	Segregating	13	4	17	3 *D*:1 *N*.
53	17	70	do	26	8	34	Do.
36	27	63	do	21	6	27	Do.
47	27	74	do	48	20	68	Do.
70	7	77	do	46	18	64	Do.
78	6	84	do	70	25	95	Do.
56	11	67	do	36	13	49	Do.
59	6	65	do	51	17	68	Do.
63	14	77	do	42	18	60	Do.
58	10	68	do	35	9	44	13 *D*:3 *N*.
69	6	75	do	30	23	53	9 D:7 N.
74	3	77	Normal with few dwarfs	39	48	87	Do.
68	2	70	do	40	43	83	Do.
57	2	59	do	29	38	67	Do.
78	1	79	do	47	32	79	Do.
80	1	81	do	15	20	35	Do.
[1] 2,966	0	[1] 2,966	All normal [1]	19	26	45	Do.
				34	32	66	Do.
				37	31	68	Do.
				8	15	23	Do.
				34	50	84	Do.
				19	32	51	Do.
				9	21	30	Do.
				16	33	49	Do.
				53	28	81	39 *D*:25 *N*.
				48	21	69	Do.
				43	27	70	Do.
				43	28	71	Do.
				45	20	65	Do.
				40	27	67	Do.
				35	16	51	Do.
				[2] 171	0	171	True-breeding dwarf.

[1] 35 rows.

[2] 2 rows.

All segregating families grown from the dwarf F_2 plants had a fairly good proportion of dwarfs, but according to expectation there should have been a preponderance of dwarf plants in all of the families

that segregated. This was the case in the majority of the families only in the Turkey-Florence × Baart cross, data for which are shown in table 4. In this cross families were obtained that indicated the expected segregating dwarf to normal ratios of 3:1, 13:3, 9:7, and 39:25. The proportion of families segregating into 3:1 and 13:3 ratios was less than the calculated values, owing to shortages of dwarf plants.

Two families in the Hussar-Hohenheimer cross were all dwarf except for one normal plant in one family and two normal plants in the other. It is probable that these plants were the results of natural crossing by normal plants. Tingey (*12*) showed that a fairly high percentage of natural crossing on dwarf plants by pollen from normal plants may occur.

DISCUSSION

When a combination of parents gives rise to a new character expression such as dwarfness in the F_1 generation, it is evident that some sort of complementary-factor relationship is responsible. It has been shown by a number of investigators that dwarfing in wheat is due to a dominant factor *D* for dwarfing and that this factor is hypostatic to a factor *I* (or *N*) for normal height. A number of normal wheats have been shown to possess the dominant *IIDD* factors, and other normal varieties the recessive *iidd* factors. To explain the production of dwarfs in F_1 from normal varieties, Thompson (*11*) assumed a third factor (*E*) for the dwarf-factor complex, which inhibits or neutralizes the *I* factor.

This scheme satisfactorily explains the general situation where F_1 dwarfs are produced from normal parents. Contrary to Thompson's point of view, however, a modified complementary-factor scheme also may be used to explain the results obtained in the crosses described by the writers and by Thompson (*11*) without assuming an inhibitor-of-an-inhibitor relationship, if his *E* factor is assumed to be a second factor for dwarfing which is not capable of producing dwarfs alone, or in the absence of *I*, but which with *D* suppresses the expression of the *I* factor. The factors *D* and *E* would thus represent different capacities for dwarfing, and the factors *IIDD* would be contributed by one parent and the *EE* factor by the other parent when dwarfs are produced in the F_1 generation. This factor hypothesis would produce the same segregations as in Thompson's scheme.

The occurrence of occasional aberrant dwarf plants in apparently normal families has been elucidated by the cytological researches of both Goulden (*5*) and Thompson. Thompson (*11, p. 346*) states that—

> In view of the difficulty in formulating a completely satisfactory genetic interpretation, one might be inclined to attribute dwarfishness to chromosome irregularity. Such a proceeding would not be justified, however, since the F_1, though dwarf, had the full complement of 21 chromosomes as did dwarfs of later generations. It seems clear that dwarfness is due to genetic factors.

He concludes that the appearance of occasional unexpected dwarf plants may very well be due to the chromosome irregularities observed.

The duplication of chromosome sets (or genoms), which is believed to have taken place in the evolution of wheat, makes it entirely possible that chromosome homology may exist to a greater or less degree among at least some of the chromosome pairs. As stated by Goulden

(*5*), "There may in some cases be sufficient affinity between chromosomes that are not strictly homologous to bring about different types of pairing." Irregular pairing would result in occasional factor combinations that would afford opportunity for expression of the dwarf factor.

The results of the experiments reported in this paper indicate the genotypic composition for dwarfing in the wheat varieties used. The three-factor combination includes the normal (or dwarf-inhibiting) factor (*I*), a dominant factor (*D*), and a complementary dwarfing factor (*E*). The only parental combinations of factors which together would yield a dwarf F_1 progeny from normal parents would be *IIDDee* and *iiddEE*.

Turkey-Florence crossed with Jenkin, Federation, Baart, and the two Arco selections 8118 and 8120 produced all dwarfs in F_1, as did Hussar-Hohenheimer crossed with Jenkin, and the Arco selections. The genotypes of Turkey-Florence and Hussar-Hohenheimer must be identical, since crosses with the same varieties gave similar results. Also the five varieties with which these two were crossed must have the same genotype for the same reason. Tingey (*12*) showed in his studies, where two factors for dwarfing were involved, that the genotype of Federation was *IIDD*. The senior writer (*4*), in his studies of dwarfs in backcrosses, found that Jenkin contained the dominant factor *IIDD* and Quality the recessive factors *iidd*. Since Baart and the two Arco strains produced the same results as Jenkin and Federation, all must have had the dominant factors *IIDD*.

The third factor, *EE*, must have been contributed by Turkey-Florence and Hussar-Hohenheimer. The genotype for dwarfing of Jenkin, Federation, Baart, and the two Arco selections apparently is *IIDDee* and that of Turkey-Florence and Hussar-Hohenheimer *iiddEE*.

SUMMARY

Dwarfs were obtained in the F_1 generation in eight crosses between normal (tall) varieties of wheat at Moscow, Idaho, in 1931 and 1932. Turkey-Florence was used in five crosses, and Hussar-Hohenheimer in three crosses. In 1931 Turkey-Florence was crossed with Jenkin, Federation, and Baart, and Hussar-Hohenheimer was crossed with Jenkin. In 1932 both Turkey-Florence and Hussar-Hohenheimer were crossed with the two selections of Arco.

The height of F_1 dwarf plants averaged from 15 to 17 inches, and that of the normal parents from 33 to 44 inches. The average weight of kernel of F_1 dwarf plants was less than that of the parents.

The segregation of dwarf and normal plants was studied in the F_2 and F_3 generations in the four crosses made in 1931. Winter-killing caused considerable loss of F_2 plants. The heaviest losses seemingly occurred among the dwarfs.

The number of normal plants, based on a three-factor difference and a ratio of 39 dwarf to 25 normal plants, was close to the expected in one of the F_2 crosses.

The expected ratio of true-breeding to segregating families from both normal and dwarf F_2 plants was verified by the F_3 results. Satisfactory fits were obtained in all crosses. In general, dwarfs in individual families were too few in number to satisfy the expected ratios.

Dwarf F_1 plants from crosses between normal varieties indicate complementary factors. To explain the results on a complementary-factor basis, a second dwarfing factor, *E*, may be assumed, which, with the established *D* factor, is dominant over *I*. The *E* factor alone, unlike the dominant *D*, is not capable of producing dwarfs in the absence of *I*.

The genotype of the varieties Turkey-Florence and Hussar-Hohenheimer, accordingly, is *iiddEE*, and that of the other varieties used *IIDDee*.

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